

Changes in carbon and nitrogen dynamics in *Sphagnum*
capillifolium under enhanced nitrogen deposition

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Declaration

I declare that this thesis and the papers within it have been composed by myself and that no part of this thesis has been submitted for any other degree or qualification. The work described is my own unless otherwise stated.

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August 2011

Abbreviations:

CL = Critical load

Dw = Dry weight

ECN = Environmental change network

EGM = Environmental gas monitor

ER = Ecosystem respiration

DOC = Dissolved organic carbon

DON = Dissolved organic nitrogen

Fw = Fresh weight

NEP = Net ecosystem productivity

NH8 = 8kg ha⁻¹ y⁻¹ of ammonium added on top of the background deposition

NH24 = 24kg ha⁻¹ y⁻¹ of ammonium added on top of the background deposition

NH56 = 56kg ha⁻¹ y⁻¹ of ammonium added on top of the background deposition

NH8PK = 8kg ha⁻¹ y⁻¹ of ammonium and PK with 1:14 ratio to N added on top of the background deposition

NH56PK = 56kg ha⁻¹ y⁻¹ of ammonium and PK with 1:14 ratio to N added on top of background deposition

NO8 = 8kg ha⁻¹ y⁻¹ of nitrate added on top of the background deposition

NO24 = 24kg ha⁻¹ y⁻¹ of nitrate added on top of the background deposition

NO56 = 56kg ha⁻¹ y⁻¹ of nitrate added on top of the background deposition

NO8PK = 8kg ha⁻¹ y⁻¹ of nitrate and PK with 1:14 ratio to N added on top of the background deposition

NO56PK = 56kg ha⁻¹ y⁻¹ of nitrate and PK with 1:14 ratio to N added on top of the background deposition

PAR = Photosynthetically active radiation

P_G = Gross photosynthesis

Restricted maximum likelihood

T = Temperature

WT = Water table

Abstract

Peatland ecosystems only cover 2-3 % of the Earth's surface but they represent significant carbon stores, holding approximately one third of the global soil carbon (C). The major peat forming genera *Sphagnum* appears to be highly sensitive to increased N availability. Many studies have shown decreased productivity of *Sphagnum* which could lead to a decrease in the amount of C stored, especially as many studies also show an increase in the decomposition rate with higher N deposition. However, the overall effects of N on CO₂ fluxes of *Sphagnum* remain unclear. The present study aimed to look at the effects of increased N on *Sphagnum* productivity, decomposition and CO₂ fluxes after long-term N additions (> 5 years) using a field experiment at Whim Moss in southern Scotland where N deposition has been manipulated employing a very realistic application coupled to rainfall since 2002. The experiment also has treatments with PK addition to test the effects of removing P and/or K-limitation. Measurements of plant tissue nutrient concentrations, visual assessments of *Sphagnum* viability, and pore water analysis were also carried out.

Nitrogen additions increased tissue N, and decreased *Sphagnum* shoot extension and productivity. Simultaneous P and K additions alleviated the effects of N on tissue N concentrations and growth, although this was only significant for shoot extension. Visual assessments correlated well with tissue chemistry and productivity; the decline in health was associated with high %N and reduced productivity. Interestingly, in the present study increased N decreased the mass loss and again when PK was added with N decomposition rates were more similar to the control. With respect to the carbon balance of the site and the sustainability of peatlands the results suggest that the negative effect of N on C assimilation may be partially offset by the reduced decomposition rates. The CO₂ measurements showed a large loss of C as CO₂ from all the *Sphagnum* plots which was exacerbated by adding N especially when the air temperature increased. The positive temperature response of ecosystem respiration with N additions suggests that in high N deposition areas climate change and subsequent temperature rises will increase C losses from bogs.

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1. Introduction

1.1. Aim of the project

In the last few centuries people have had a big impact on practically all natural ecosystems and their functioning. Peatlands are ecosystems where plant remains accumulate as peat, due to low temperatures and high water tables. They cover only a small land area but they are extremely important as the accumulation of peat provides a natural carbon sink; moreover, they have a specialised fauna and flora which makes them important in conserving the world's biodiversity (Gorham 1991; Wheeler 1993). The UK has a relatively large proportion of the world's peatlands especially for the case of some particular peatland types, for example blanket bogs and raised bogs (Lindsay *et al.* 1988). Peatlands occupy around 1.3 million hectares in Great Britain, although their area has declined due to increasing demand for and changes in land use (Lindsay *et al.* 1988). Apart from these deliberate losses, their sustainability has also been threatened indirectly by man's activities which through combustion processes and intensive agriculture have led to the enhanced deposition of atmospheric pollutants, especially sulphur (S) and nitrogen (N) (Galloway *et al.* 1995; Galloway & Cowling 2002). This has been an acute problem in certain parts of the UK, mainly in the Pennines of England, where the increased N and S from industrialised areas has had a detrimental effect on peatlands through damage to *Sphagnum* species (Ferguson *et al.* 1978; Ferguson & Lee 1979). Deleterious effects of S are arguably less of an issue today due to the dramatic reductions in S emissions which have fallen by circa > 90% from what they were 35 years ago (Fowler *et al.* 2009).

Enhanced N deposition, however, remains a relatively unquantified concern for bogs, especially since N is, second to C, the major growth limiting nutrient for these ombrotrophic ecosystems and is one of the key drivers determining carbon cycling. Enhanced N-deposition is a widespread phenomenon, likely to affect all of the UK's peatlands (Fig. 1.1). Most of the UK peatlands are situated in Scotland and the only

ones close to pristine conditions are found there (Lindsay 1993). Although N deposition to the majority of peatland areas is at the low end of the UK N deposition range, it still exceeds the Critical Load (CL) for bogs ($5\text{--}10 \text{ kg N ha}^{-1} \text{ y}^{-1}$) for ~ 50% of Scottish peatbogs (UNECE 2003; Fig. 1.1). Clear detrimental effects of CL exceedance have been seen in Dutch bogs where *Sphagnum* has been replaced by more nitrophilous species (Limpens & Berendse 2003a). The potential for enhanced N deposition *per se* to deleteriously affect the sustainability of similar habitats, wetlands and heathlands has been recognized across Europe since the eighties (Roelofs 1986). However, most of the evidence has come from laboratory or transplant based studies with mainly *Sphagnum* species (Press & Lee 1986; Woodin & Lee 1987a), or more recently looking for relationships between N deposition and *Sphagnum* along gradients of N deposition, which also tend to be confounded with other environmental variables (e.g. temperature) (Bragazza *et al.* 2006). Very few studies have evaluated the long-term responses of bog ecosystems to known N inputs where influences of these other environmental variables can be controlled. Such studies can really only be addressed with manipulation experiments.

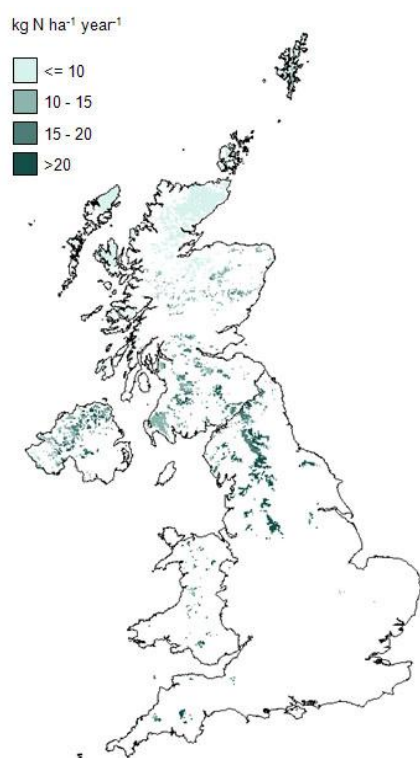


Figure 1.1. The overall N deposition laid over bog distribution in Britain.

The overall focus of this research was to capitalize on a long running N manipulation study at Whim bog in the Scottish Borders in order to quantify the effects of long-term increased N deposition, and in particular show whether the form of N deposition, reduced versus oxidised N, makes a difference to the responses of *Sphagnum capillifolium* (Ehrh.) Hedw., a common peat-forming species in the UK. The overall aim of the study was to examine the field responses of *S. capillifolium* to enhanced N deposition with respect to its carbon economy and identify some of the risks posed by N to the sustainability and functioning of Scottish peatlands. The following literature review expands on the subject areas introduced above, in order to highlight the potential research issues and their relevance to peatlands in Scotland, where most of the UK's peat resources are located, and the services they provide with respect to conservation, biodiversity and carbon sequestration. The underpinning premise to this project was: 'since most peatlands owe their very existence to the genus of plants called *Sphagnum* we can gain an insight into the effect of N on the sustainability of peatlands by focussing on the responses of this keystone genus'.

1.2. Peatlands

Northern peatlands cover 2-3% of the global land surface, which approximates to c. 353 Mha worldwide; most of them are situated in the northern hemisphere (Gorham 1991; Moore 2002; Rydin & Jeglum 2006).

In general, peatlands can be divided into two different types, fens and bogs. Minerotrophic fens and bogs receive nutrients both from atmospheric deposition and from surface run-off or ground water whereas ombrotrophic bogs receive **all** their moisture and nutrients from the atmosphere which is also reflected in their lower nutrient and pH status (Wheeler & Proctor 2000; Charman 2002; Rydin & Jeglum 2006). It has also been suggested that because it is extremely difficult to determine the hydrology of a peatland, acidity and low nutrient content could be used to signify the definition of a bog (Wheeler & Proctor 2000). Peatlands can develop through three different routes, by: (i) terrestrialisation where a lake becomes overgrown and

turns into a fen and after this into a bog or (ii) paludification where peat starts to develop over soil in suitable conditions and (iii) by primary formation where peat is formed directly on wet soil (Sjörs 1983). Bogs have lower nutrients status, for example total N and phosphorus (P) pools are lower than in fens, but at the same time the turnover of nutrients is more rapid (Bridgham *et al.* 1998; Wheeler & Proctor 2000). Bogs are also very acid with a low pH (< 4.5) and a low base status, (Wheeler & Proctor 2000; Charman 2002; Rydin & Jeglum 2006). A prerequisite for ombrotrophic bogs to develop is a surplus of water. Moisture conditions, i.e. the relationship between rainfall and evapotranspiration, the regularity of water supply and the temperature because it affects evapotranspiration are all important factors (Lindsay 1995).

The classification of peatlands is based on different features, e.g. hydrology, vegetation or the landform of the area and different systems are used in different countries (Wheeler & Proctor 2000; Rydin & Jeglum 2006). The most common types of peatlands in the UK are raised bog and blanket bog, which develop on different places based on the specific requirements (Lindsay 1993; Wheeler 1993) (Fig. 1.2).

Blanket bogs occupy a variety of topographical situations but they have very particular circumstances where they occur (Wheeler 1993). Lindsay *et al.* (1988) have suggested the following prerequisites for the formation of blanket bogs: minimum of 1000 mm rainfall per year, minimum of 160 wet days (> 1 mm rain), mean temperature of <15°C for the warmest month and minor seasonal fluctuation in the temperature. In the British Isles, 7.5% of the land area is covered by blanket bogs representing 10 to 15% of the global total and together with Ireland represents the largest resource of blanket bogs in Europe (Lindsay *et al.* 1988; Steiner 1997; Tallis *et al.* 1997). Only 3% of all the peatlands of the world are classified as blanket bogs (Tallis *et al.* 1997). In the UK there is estimated to be 2,195,200 ha of blanket bog habitat, of which 1,759,000 ha is in Scotland (Joint Nature Conservation Committee 2007a and b). Blanket bogs in the UK are dominated by *Sphagnum* mosses, other bryophytes, sedges, dwarf shrubs and occasionally lichens (Joint Nature Conservation Committee 2009).

Raised bogs are mainly restricted to lowland areas, unlike blanket bogs which are at their southern limit and are found at higher altitudes (>300m) (Fig. 1.2). Raised bogs represent individual domes of peat which are very ombrotrophic, as there is no influence of additional nutrients coming from flushing in (lateral transport) or salt spray as in blanket bogs (Lindsay 1993). They occur in areas with lower rainfall and are totally dependent on rainfall for their water and nutrients (Lindsay 1993). Their peat deposits also tend to be much thicker due to less decomposition or compression than peat in blanket bogs (Lindsay *et al.* 1988, 1993). Raised bogs, however, are a lot less common in Scotland than blanket bogs (Lindsay 1993); in total in the UK there is 12,941 ha of active raised bogs of which 3,325 ha is in Scotland (Joint Nature Conservation Committee 2007a and b).



Figure 1.2. Areas where blanket bogs (left) and most lowland raised bogs (right) are found in Scotland (from Scottish Natural Heritage 1995).

1.2.1. *Sphagnum* mosses

There are approximately 150 to 200 *Sphagnum* species in the world, and most of them are found in the northern hemisphere (Clymo 1997). *Sphagnum* is the most typical, and in many ways the most important, plant genus in ombrotrophic bogs. It has been suggested that more C is stored within live, necrotic and decomposing *Sphagnum* mosses than in any other plant genus, including trees (Clymo & Hayward 1982; Clymo 1997).

They can be very productive under suitable conditions; on a British blanket bog productivities of $150 \text{ g m}^{-2} \text{ y}^{-1}$ (hummocks), $500 \text{ g m}^{-2} \text{ y}^{-1}$ (lawns) and $800 \text{ g m}^{-2} \text{ y}^{-1}$ (pools) have been recorded (Clymo 1970; Clymo & Reddaway 1972; Clymo & Hayward 1982). *Sphagnum* mosses grow indefinitely from the capitulum (the apical portion of the stem with dense branching) while continuously decomposing from the lower parts (Clymo & Hayward 1982). They have been called ecological engineers because they have morphological and physiological properties, which create nutrient poor and acidic conditions that favour them (Van Breemen 1995; table 1.1).

Sphagnum mosses have a high cation exchange capacity; they remove cations from the solution replacing them with hydrogen ions (Clymo 1963; Clymo & Hayward 1982). Uronic acids that function as exchange sites for cations constitute between 10 and 30% of the dry mass of *Sphagnum* (Spearing 1972; Clymo & Hayward 1982). Cation exchange lowers the pH of the peatland and in this way the acidity created by *Sphagnum* can inhibit the growth of other plants or microbes (Clymo 1963; Clymo & Hayward 1982; Van Breemen 1995) and decrease both its own rate of decay and that of neighbouring plants (Verhoeven & Toth 1995). Thus, species of *Sphagnum* create a suitable environment for their own growth at the expense of other life forms, and bogs usually have a low cover of vascular plants with *Sphagnum* species forming the bulk of living and dead biomass (Clymo & Hayward 1982; Van Breemen 1995).

Table 1.1. Specific properties of *Sphagnum* that increase its fitness in the short term and in the long term (through promoting peat formation) (according to Van Breemen 1995).

Property	Short-term benefit	Long-term benefit
Organochemical composition	Anti-herbivory action	Peat formation
Microstructural (hyaline cells)	Water conservation	Finely porous, impermeable peat
Macrostructural (pendent branches)	Capillary water supply	Collapses easily to dense peat
High nutrient retention	Efficient nutrient use	Low nutrient supply to vascular plants

Sphagnum mosses, like most mosses, take up water as well as nutrients, over their entire surface. *Sphagnum* mosses are adapted to withstand drought; up to 80% of their volume is made up of hyaline cells which store water and they can withstand a drought of several weeks and still recover when it rains again (Van Breemen 1995; Clymo 1997). The hyaline cells are emptied during droughts, which gives the plants a whitish appearance and causes higher reflectance (Van Breemen 1995). Although each plant cannot actively move water like vascular plants, capillary action in the dense tussocks moves water from underlying layers to the upper, actively growing layers (Clymo & Hayward 1982; Rydin & Jeglum 2006). Because they acquire all their water from atmospheric inputs they make good bioindicators for hydrological and chemical changes in the atmosphere, and have been used to test for the presence of heavy metal pollution in the atmosphere over the British Isles (Ferguson *et al.* 1984; Lee & Studholme 1992).

1.2.2. Carbon accumulation in peatlands

In peat forming systems the high water table leads to a diplotelmic structure where an oxic layer, the acrotelm, is situated above an anoxic layer, the catotelm (Ingram 1983) (Fig. 1.3). In peatlands the growth and decomposition occur mainly in the surface 50 cm, *i.e.* the acrotelm (Ingram 1983; Clymo 1984). Decomposition is already slow in the acrotelm but it is further slowed down in the anoxic conditions of the catotelm (Clymo 1984). The decomposition rate of *Sphagnum* in the anoxic layer is 100-1000 fold slower than in the oxic layer (Clymo 1997). As a consequence in peatlands the rate of carbon (C) uptake through primary production exceeds the rate of decomposition (Clymo 1983). It is specifically the slow decomposition rate, rather

than high productivity, that determines the peat and C accumulation rate (Clymo 1983; Clymo 1997); even so, up to 90% of the C sequestered in primary production is released back to the atmosphere as carbon dioxide (CO₂) (Clymo 1984). However, it has been estimated that despite the huge proportions of the fixed C that are lost, about 455 Gt of C has been stored in boreal peatlands since the last glacial period; this is equivalent to one third of the global soil C pool (Post *et al.* 1982; Gorham 1991). The current annual accumulation rate in northern peatlands is thought to be 0.07 Gt of C (Clymo *et al.* 1998), although the amount of C sequestered can vary between years, depending on weather conditions (Alm *et al.* 1999; Roulet *et al.* 2007). However, the slowly decomposing plant matter in the catotelm under anoxic conditions produces methane (CH₄), which diffuses back to the atmosphere (Clymo 1983; Clymo 1984; Rydin & Jeglum 2006). Accordingly, pristine peatlands act as sinks for CO₂ and sources of CH₄ (Clymo 1983; Alm *et al.* 1997). Consequently, any disturbance to peatland ecosystems will have significant repercussions for C balance on a global scale and will potentially affect climate change (Moore 2002). Likewise, climate change may, through increasing temperatures and/or falling water tables, alter the C balance of peatlands and might turn them into net emitters of carbon to the atmosphere (Gorham 1991; Moore 2002), either as CO₂ or as CH₄. The accumulation of C as peat also locks up other nutrients and peatlands play an important role in other global biogeochemical cycles, e.g. nitrogen (N).

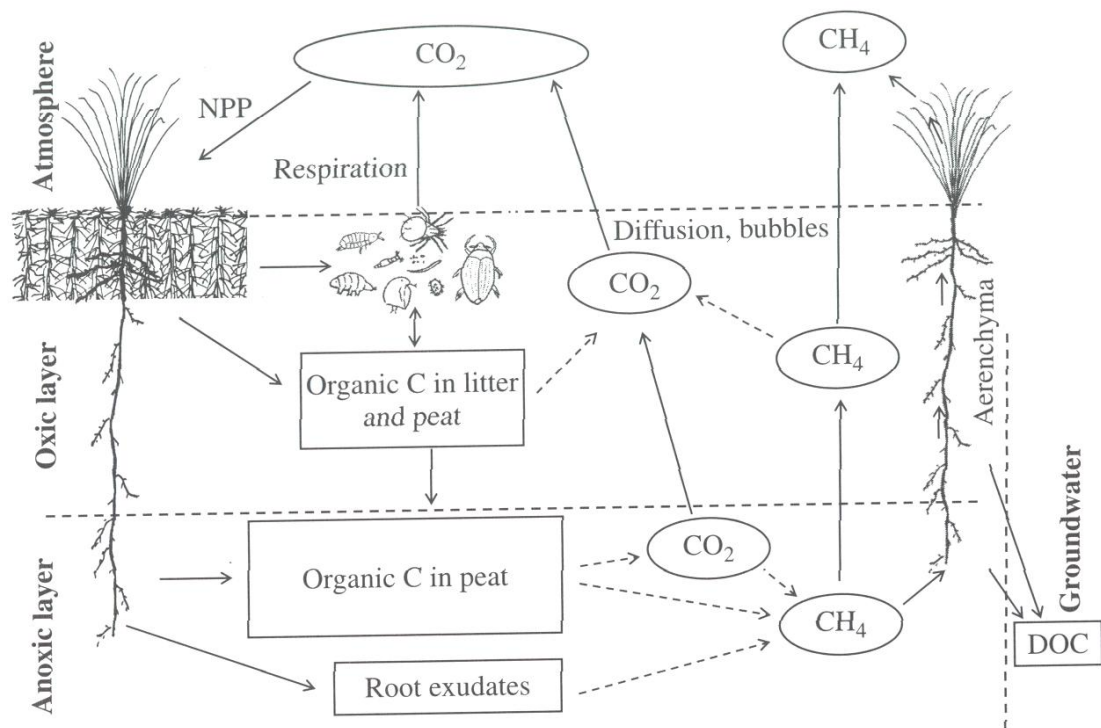


Figure 1.3. Carbon cycle and peat formation in peatlands. Dashed arrows are microbial processes and circled symbols are gaseous forms and boxes are solid forms (from Rydin & Jeglum 2006).

1.2.3. Nutrient cycling in peatlands

Peatlands in general have scarce resources of plant available nutrients because these nutrients are sequestered within the peat and mineralization rates are low (Rydin and Jeglum 2006). Ombrotrophic bogs are especially nutrient poor because they rely on the atmosphere for nutrients, unlike fens which are flushed with ground water.

Throughout the boreal and temperate zone in peatlands the most important growth (limiting) elements for plants are N and P, but most usually N (Hayati & Proctor 1991; Aerts *et al.* 1992; Hoosbeek *et al.* 2002). Nutrient limitation occurs when one or more nutrients are not sufficiently available for plants to attain the maximum growth afforded by the environmental conditions (Chapin *et al.* 1986). Accordingly, the productivity of an ecosystem normally increases when there is an increase in the amount of limiting nutrient. However, if the plants are adapted to low nutrient availability, as in bogs, they might not respond to increased amount of N by increasing their growth and furthermore more nitrophilous species that benefit from

the increased nutrient supply, e.g. grasses, are enabled to outcompete and replace them (Chapin *et al.* 1986; Limpens *et al.* 2003a).

In strongly acidic environments such as ombrotrophic bogs ammonium (NH_4^+) tends to be the dominant form of mineral N in the soil solution (Waughman 1980; Hemond 1983; Hayati & Proctor 1991). Therefore, it is perhaps not surprising that uptake studies conducted in the laboratory indicate higher uptake rates for ammonium-N than for nitrate-N (NO_3^-) (Jauhiainen *et al.* 1998). *Sphagnum* has no means of restricting NH_4^+ uptake in response to increasing availability and the accumulation of ammonium leads to reduced activity of nitrate reductase following reduced ability of *Sphagnum* to assimilate nitrate (Woodin and Lee 1987b). *Sphagnum* has developed many ways to secure the nutrients in the nutrient poor conditions in bogs and they use the scarce amounts efficiently. They accumulate inorganic N through influx to the chlorophyllous cells following adsorption to exchange sites on the cell wall (Clymo & Hayward 1982). The N is derived from wet and dry deposition, mineralization and reabsorption from the lower parts of the stem (Aldous 2002a and b; Robinson 2002). *Sphagnum* mosses can relocate significant amounts of N, P and K from old, senescing parts to the new growing capitulum although the process requires the water table to be high for vertical transport (Rydin & Clymo 1989; Aldous 2002b). *Sphagnum* can also acquire N from fixation by bacteria, but this does not constitute a large part of the N budget (Waughman & Bellamy 1980; Hemond 1983; Aldous 2002b). Because N is generally available in scarce amounts, deposited inorganic nitrate and ammonium, are rapidly taken up by the vegetation and micro-organisms, so that most N in bogs is immobilised in organic forms (Urban & Eisenreich 1988; Urban *et al.* 1988). For other macronutrients, phosphorus (P) and potassium (K), bogs depend almost totally on the internal cycling (Bridgham *et al.* 2001), although in oceanic areas some is aurally deposited from sea spray.

The nutrient poor conditions of bogs have led to development of mechanisms for high nutrient use efficiency (NUE). The species composition and ecosystem processes are adapted to these N-poor conditions and therefore the plants of pristine bogs are only capable of competing in N-limited conditions (Emmett 2007). Species

characteristic of these nutrient impoverished environments have evolved to conserve nutrients rather than exploit them through upregulation of assimilation and growth (Emmett 2007). Because *Sphagnum*, along with all mosses, efficiently capture nutrients from the atmosphere and are sensitive to changes in nutrient balance they can be used as indicators for changes in atmospheric N deposition (Pitcairn *et al.* 1995).

While atmospheric N deposition can relieve the N limitation in ecosystems such as bogs it does not address the availability of other nutrients such as P and K which are often co-limiting (Hoosbeek *et al.* 2002). Their demand also increases with increasing N deposition. The N/P and N/K ratios can be used as indicators of the limiting nutrients in the ecosystem. There is a general agreement that if the N/P ratio is < 14 the ecosystem is N-limited, if it is between 14 and 16 it is co-limited by the two nutrients and if it exceeds 16, the ecosystem is P-limited (Koerselman & Meuleman 1996; Hoosbeek *et al.* 2002; Rydin & Jeglum 2006). Hoosbeek *et al.* (2002) suggested a similar relationship for N and K, so that when the N/K-ratio is below 1.2 it indicates N-limitation, ratio $1.2 < N/K < 1.4$ indicates N and K co-limitation and ratio > 1.4 indicates K-limitation. However, Bragazza *et al.* (2004) suggested considerably higher ratios of N:P ratio of ~ 30 and N:K ratio of 3.3-3.4 as the limits for changing from N limitation to P or K limitation in bog ecosystems. When growth is restricted by the availability of nutrients other than N mosses such as *Sphagnum*, which have no way of adjusting their N uptake to match demand (Clymo & Hayward 1982; Lamers *et al.* 2000), the N tends to accumulate with a range of detrimental effects (Lamers *et al.* 2000; Limpens & Berendse 2003b).

In addition to nutrients, pH is an important determinant of species composition in peatlands (Glaser *et al.* 1990; Vitt & Chee 1990; Anderson *et al.* 1995). Essential chemical and biological reactions and functions, as well as the availability of some nutrients, are all affected by pH (Lucas & Davis 1961; Verhoeven *et al.* 1990). However, it seems that the importance of pH lies in its effects on nutrients; Chapin *et al.* (2004) found that pH effects were strongly related to changes in nutrient

availability. As well as nutrients and pH, water level and climate are important factors structuring the plant community in peatlands (Vitt & Kuhry 1992).

1.3. Conservation value of peatlands

The UK contains a large proportion, of the global peatland area; a large part of it is blanket bog (Lindsay *et al.* 1988). While raised bogs are also common in other parts of the world, British raised bogs are special because of the highly oceanic climate which makes them structurally and floristically different from other raised bogs (Joint Nature Conservation Committee 1994). Besides being globally important, the ombrotrophic bogs occur within many of the last remaining wilderness areas in the UK and provide an important refuge for many species of plants and animals (Barkham 1993). Because peat based bogs can represent thousands of years of organic matter accumulation they are among Britain's most ancient natural/semi-natural ecosystems.

The importance of peatlands is now recognised both internationally and in the UK. Peatland vegetation types are important habitats under the Ramsar convention (2006) and make up a part of the Ramsar designated sites in the UK. Active raised bogs and blanket bogs are both included in the Annex 1 of the Habitats and Species Directive (92/43/EEC) which requires Member States to take measures to maintain at, or restore natural habitats and wild species to, favourable conservation status by designation of Special Areas of Conservation (Scottish Office 2000). Active blanket and raised bogs are also priority habitats for conservation and restoration under the UK Biodiversity Action Plan (1994) (UK Biodiversity group 1999).

There are both animals and plants that are dependent on these ecosystems. Two thirds of British dragonflies breed in bogs and there are 11 species which rarely occur anywhere else (Brooks 1997; Stoneman 1997). Blanket bogs are internationally important habitats for birds of prey (Thompson *et al.* 1995) and support a significant portion of the total populations of some bird species in Europe (Stroud *et al.* 1990; Joint Nature Conservation Committee 1994). Bogs are also

important for many plant species and have some highly specialised species that are adapted to the waterlogged and nutrient poor conditions (Joint Nature Conservation Committee 1994; UK Biodiversity group 1999). An example of an uncommon species in Scotland living in peatlands is *Andromeda polifolia* (Jacquemart 1998).

In addition to their biodiversity value, bogs provide many other ecosystem services (Keddy *et al.* 2009). They act as natural filters providing clean water and in many systems stabilise the water levels and prevent flooding (Keddy *et al.* 2009). They are very important archives of historical information due to the fact that the accumulated peat is very acid and preserves anything that is stored within it for thousands of years. They store evidence of their own history and climatic changes as well as archaeological material (Rydin & Jeglum 2006). Peatlands also have a recreational value for people (Barkham 1993). Arguably the most important ecosystem service that peatlands offer, especially currently with the threat of climate change, is storing C and acting as C sinks. For these many reasons it is important that peatlands are being conserved or if they have already been degraded, they are restored back to functioning, carbon accumulating ecosystems.

1.4. Anthropogenic nitrogen deposition

N forms an important part of the global biogeochemical cycle. Natural sources of oxidized N include lightning and nitrification of mineralized N *i.e.* NH_4^+ in the soil. Natural sources of reduced N include biomass decomposition, mineralization and animal waste (NEG-TAP 2001).

In the last few decades N deposition has been increasing in many areas in the northern hemisphere due to anthropogenic activities; industrialization, intensification of agriculture and fossil fuel burning have led to a three to five-fold increase in N emissions in the last century (Reay *et al.* 2008). The natural deposition of N is difficult to estimate because the measurements started after industrialization, but recent anthropogenic inputs have been estimated to contribute roughly 50% of total

N emissions (Galloway & Cowling 2002; Fowler *et al.* 2005) exceeding that fixed biologically (Galloway *et al.* 1995; Vitousek *et al.* 1997).

In Europe, the least polluted areas are in Scandinavia and northern Scotland and the most polluted areas are found in central Europe, especially in the Netherlands (Asman *et al.* 1988). In the UK emissions of oxidized N between the years 1986 and 2006 were between 910 and 386 kt declining over time whereas the emissions of reduced N between 1990 and 2005 between 391 and 326 kt have remained fairly constant (Fowler *et al.* 2005; Malcolm 2010). Because of the differences in atmospheric lifetime most of the emitted reduced N remains in the UK compared with oxidized N which is mostly (85%) exported and deposited outwith the UK (NEGTAP 2001). The total annual deposition of N in Britain has been estimated to be 400 kt, which averages to about $17 \text{ kg ha}^{-1} \text{ y}^{-1}$; the deposition is split between oxidized N (43%) and reduced N (57%) (NEGTAP 2001; Malcolm 2010). The overall deposition in the UK is declining but the dry deposition is declining more rapidly than the wet deposition, which leads to change in the partitioning of the deposition, i.e. a bigger portion of the deposition is wet deposition (Fowler *et al.* 2005). The deposition of dry versus wet N is not equally distributed over the country because high rainfall areas receive more wet deposition of N although they have lower concentrations of the pollutants (Fowler *et al.* 2005; NEG-TAP 2001).

Due to all these factors the lowlands experience hotspots of ammonia while the uplands receive most N in precipitation, mostly dominated by reduced N (Fowler *et al.* 2005; Malcolm 2010). The threat to semi-natural ecosystems from dry, gaseous N deposition tends to be greatest close to the source. However, these gases also form the basis of regional wet deposition when transported huge distances as aerosols and particles, until they are washed out in precipitation. In addition, although N deposition has been assumed to decline, measurements show little change in N deposition at the remote UK sites where most bogs occur (Fowler *et al.* 2005). In order to regulate N emissions in response to the recognized threats enhanced N deposition poses in particular to semi-natural ecosystems, the concept of Critical Load (CL) was developed as a policy tool. Critical N loads represent a quantitative

estimate of an exposure to N deposition as NH_x and NO_y below which harmful effects in ecosystem structure and function do not occur according to present knowledge (Nilsson & Grenfelt 1988). The approaches for estimating the CL include both empirical studies and models. The empirical approach is based on observations of changes in receptors and draws on evidence from experiments, both controlled and field manipulations. Based on the above together with expert judgement the critical load for raised and blanket bogs has been estimated to be $5\text{--}10 \text{ kg ha}^{-1} \text{ y}^{-1}$, the range taking account of factors such as P limitation which can ameliorate the impact of N such that the system can tolerate the higher N load (UNECE 2003).

1.5. Current state of UK peatlands

The current status of bogs in the UK is worse than anywhere else in Europe (Steiner 1997). Less than 5% of the total original area of raised bogs in the UK remains; in a survey of almost 1000 raised bogs throughout the country, not a single one was found to be free from anthropogenic disturbance (Lindsay 1993) (Table 1.2). Steiner (1997) found that in England there are no undamaged bogs left and although in Scotland the situation is marginally better, no undisturbed bogs are to be found in Scotland either, although a number of sites are in fairly good condition. More recently surveys for the Common Standards Monitoring for Designated Sites: First Six Year Report (Williams 2006) indicate that 54% of blanket bogs and 21% of lowland raised bogs are in favourable condition. Because only a very few bogs in good condition remain they are very susceptible to random incidents such as fire, which could destroy them (Lindsay 1993). In addition, due to the small number of bogs left, they are very fragmented making them hydrologically unstable (Lindsay 1993).

Table 1.2. Present day remaining area and SSSIs of near-natural bog vegetation as a proportion of the area of land under soils formed from raised bogs (from Joint Nature Conservation Committee 1994).

	Amount of land with raised bog soils		Amount of land with near natural bog vegetation	
	Area (ha)	No. of SSSIs	Area (ha)	No. of SSSIs
England	37,413	210	493 (1%)	15 (7%)
Scotland	27,892	851	2,515 (9%)	129 (15%)
Wales	4,086	21	818 (20%)	6 (29%)
Total	69,391	1,082	3,826 (6%)	150 (14%)

1.5.1. Threats to the UK peatlands

Anthropogenic activities represent a major threat to bogs through for example grazing, burning, drainage, peat extraction, forestry, agricultural improvement (fertilisation and liming), enhanced atmospheric deposition, recreation tracks leading to erosion and clearing for wind farms or communication masts (Barkham 1993; Coupar *et al.* 1997; Joint Nature Conservation Committee 2007a; Williams 2006). Some anthropogenic induced change have led to irreversible losses of peatlands e.g. where the majority of peat has been removed or drained over decades (Lindsay 1993). Currently peat harvesting is the most significant threat to the remaining lowland raised bogs (Barkham 1993; Lindsay 1993). Nowadays peat is removed under licence, and where this occurs, especially prevalent in lowland raised bogs, they are drained and stripped of surface vegetation. Today licences are only granted under strict operating and post removal conditions whereby sites have to be restored or at least re-vegetated. In Scotland afforestation led to large-scale drainage of peatlands especially in the Flow country, but these activities are now being reversed through drain blocking (Johnson S., pers. comm.).

Burning of *Calluna* and grass vegetation on peatlands is common practice to provide habitat more suitable for grouse, a wild bird which along with deer forms the basis of a valuable commercial sporting industry. Regular burning of peatland may lead to loss of *Sphagnum* and increase of vascular plants (Lindsay *et al.* 1988; Williams 2006). However, there are clear guidelines set out in the Heather and Grass Burning

codes in England and Wales and the Muirburn code in Scotland on where and when burning should not be done on blanket bog (DEFRA 2007; Scottish Government 2008). Sites that have been burnt are also susceptible to invasion by trees and in such areas scrub removal is required to prevent this; this is a problem on raised bogs but on blanket bogs ongoing burning and high grazing intensity prevents trees from establishing (Wheeler 1993). Peat erosion is also widespread throughout the peatlands of the UK (Mackay 1997; Lindsay *et al.* 1988). It leads to drying of the bog and changes in the vegetation, mainly from spreading of graminoid species (Mackay 1997). To prevent erosion it is important to maintain intact vegetation cover (Mackay 1997). However, both sheep grazing and burning increase susceptibility to erosion. Wind farms have emerged as a new threat to bogs in recent years. Bogs have been found to be very sensitive to the impacts of wind farming and care should be taken during construction (Natural England 2010). It has also been suggested that degraded peatland sites could be restored at the same time with building wind farms to mitigate the harmful effects (Natural England 2010). The land use in the surrounding area is also crucial for the conservation of bogs, both as a source of pollutants e.g. intensive agriculture (ammonia) and as a source of invasive, undesirable plant species and through effects on the water table (Barkham 1993). Likewise fragmentation can restrict certain species from migrating to new areas and can put the survival of the full species complement of the biotope at risk (Joint Nature Conservation Committee 1994; Wilson & Provan 2003).

For blanket bogs the activities that have most contributed towards unfavourable conditions, as recorded in the Common Standards Monitoring (Williams 2006) are over-grazing and burning. Also water management and a lack of conservation have contributed (Williams 2006). The main causes for unfavourable conditions of raised bogs are water management (drainage) and neglect. Other causes are invasive species and, contrary to blanket bogs, under-grazing (Williams 2006).

The majority of these activities involve active intervention and cause changes which are likely to be immediately visible. In these cases the reasons for the degradation are easily identified enabling relatively straight forward regulation. Threats from

enhanced N deposition however, while potentially just as far reaching, are likely to be much more subtle and more difficult to see and identify, taking years or even decades to manifest themselves. In addition there might be several sources of pollution which make identifying and regulating them difficult. It is crucial that we improve our understanding of the scale of the N threat to bogs so that we can work effectively towards their protection and improving their state commensurate with meeting our Habitats Directive (92/43/EEC) and Biodiversity Action Plan commitments (UK Biodiversity Group 1999).

1.6. Actions for improving the conservation of peatlands

The EC Habitats Directive (92/43/EEC) and UK Biodiversity Action Plan (UK Biodiversity Group 1999) provide the policy drivers towards improving the conservation status of raised and blanket bogs in the UK through improved management and restoration. For the areas that have already been damaged, restoration is a possible way of recovering the wildlife and C sink function of active bogs. While restoring damaged peatlands could potentially provide a C sink the effects on the overall C balance of the peatland remain unclear, since although active peatlands are sinks of C they are also sources of methane, a highly significant radiative forcing, greenhouse gas. Further research is required to establish the overall C balance of bog restoration projects, to establish the effectiveness of this method for storing C. It also needs to be kept in mind that restoration processes can take decades or more.

1.7. Research questions and hypotheses

Despite a considerable research effort there are still many gaps in our understanding of the effects of enhanced reactive N deposition on *Sphagnum* species and thus the sustainability and functioning of peatland ecosystems. In Scotland where the resource is both large and of very high conservation value we need to understand the effects of long-term low level additions. Increased N deposition has been shown to

affect *Sphagnum* productivity, decomposition and CO₂ fluxes (Lamers *et al.* 2000; Aerts *et al.* 2001; Berendse *et al.* 2001; Limpens *et al.* 2003a; Limpens & Berendse 2003a&b; Saarnio *et al.* 2003; Bragazza *et al.* 2006; Bubier *et al.* 2007). However, no studies have been done in Scotland and in general most of the studies that have been carried out elsewhere have been relatively short-term. More importantly the form of N has not been investigated in the field in a realistic simulation where the N dose is achieved through a realistic scenario, *i.e.* at high frequency and using realistic concentrations. The study was conducted on a lowland raised bog in Scotland. Restricting the study to one site is not ideal because local circumstances can confound the responses. Since the manipulation experiment was unique, this study incorporated two northern Environmental Change Network sites where background N deposition and climate have been monitored since 1994 and in the case of Moorhouse there are many studies of peatland responses to draw on and make comparisons with (for example Clymo 1973; Clymo & Reddaway 1974; Cundill *et al.* 2007).

The overall aim of this research project was to quantify effects of increased N deposition on carbon and nutrient use in *Sphagnum capillifolium*, a common hummock species in peatlands as a surrogate for peatland sustainability. Particular questions to address include:

- Does N still increase growth rate after 5 years of application?
- Are the N thresholds for nutrient concentrations found in previous studies similar when the N additions are sustained over 5 years?
- Do any of the found responses of *Sphagnum* to N offer a possible tool for identifying bogs that have been impacted by excess N?
- Does N increase decomposition rates and the ability of bogs to sequester C?
- How does enhanced N deposition affect C assimilation via photosynthesis and loss through respiration?
- What is driving the effects on decomposition, changes in litter quality or some other factors?
- Does climate influence the N response either through the availability of water or temperature?

- Are N responses in bogs tempered by the availability of the potentially co-limiting nutrients P and K and do additions of these nutrients offer a means of managing detrimental N effects in certain circumstances?
- Does the form of N, reduced versus oxidised, in wet deposition affect the responses of *S. capillifolium*?
- Is there a threshold N dose when changes can be seen?

Hypotheses underpinning these questions:

1. Although ammonium is the most abundant mineral N form in bogs, excess ammonium can be phytotoxic: an effect of the form of N is expected and effects of ammonium will be more detrimental than those of nitrate for a given N dose.
2. Oxidised and reduced N will have different effects on biological processes mediated by differences in pH and base cations.
3. N concentrations > 1.2% are indicative of a decline in *Sphagnum* vitality.
4. As the availability of reactive N increases, differences in N concentrations between the stem and capitulum will disappear.
5. Responses to experimental N additions are similar to those measured under natural conditions where deposition is quantified.
6. The responses to N deposition are not linear and are strongly affected by co-limiting nutrients.
7. Increased N availability will restrict growth because although rates of photosynthesis are enhanced the carbon costs of maintenance and repair will exceed the additional C fixed.
8. N will increase rates of decomposition of labile carbon and in balance C sequestration will decline.
9. N will increase mass loss and DOC through changes in litter quality.
10. PK amendments will mitigate the effects of increased N deposition and may offer a management tool in circumstances where N deposition is unlikely to decrease.

2. Overview of thesis

To be able to assess the total N and C budget of *Sphagnum* at the study site the research was divided into sections to answer specific questions (Fig. 2.1). These sections are written up as papers and the contributions of individuals to the different papers is detailed in Table 2.1.

Table 2.1. Contribution of individuals to different papers.

	I	II	III
Original idea	SK, LS	SK, LS	SK, LS
Study design	SK, LS, IL	SK, LS, IL	SK, LS, IL
Data gathering	SK	SK	SK
Paper preparation	SK, LS	SK, RR, LS	SK, JG

SK: Sanna K. Kivimäki. LS: Lucy J. Sheppard, IL: Ian D. Leith, RR: Robert M. Rees, JG: John Grace

Three study sites were used for carrying out the field measurements for the whole project; the main study site was Whim Moss near Edinburgh in southern Scotland and additional sites were at Moor House in northern England and the Cairngorms in the Eastern Highlands of Scotland. All the sites are extensively used for scientific research (see Chapters 3.1.1 and 3.1.2). All the material and methods used are described in Chapter 3 and the results are presented in Chapters 4-7. The results are drawn together and discussed in Chapter 8.

Paper I is based on the plant tissue nutrient, shoot extension and productivity measurements done by the broadly used cranked wire method. The measurements were carried out in all three study sites between October 2006 and September 2009.

Paper II focuses on the results from litter bags studies measuring decomposition rate and pore water measurements done with Rhizon samplers. The litter bag measurements were done at all three study sites between April 2007 and April 2009 but pore water was only collected at Whim during year 2008.

Paper III concentrates on the CO₂ chamber measurements carried out at Whim during year 2008. Due to time constraints only control and high N treatments were used for measuring CO₂ fluxes.

The specific aims of the study were to:

- 1) examine the effects of different N loads and forms (reduced and oxidized) on the productivity of *Sphagnum capillifolium*, measured by growth (Paper I) and by CO₂ fluxes (Paper III),
- 2) examine the effects of different N loads and forms on the decomposition of *S. capillifolium*, measured by litter bags (Paper II) and CO₂ fluxes (Paper III),
- 3) estimate the effects of different N loads and forms on the C and N balance of patches of *S. capillifolium* (Papers I to III and Chapter 7), and
- 4) assess the effects of P and K on all these N mediated modifying effects above when added with N (Papers I-III and Chapter 7).

Tables and figures are labelled starting from one in every chapter and prefixed with the chapter number.

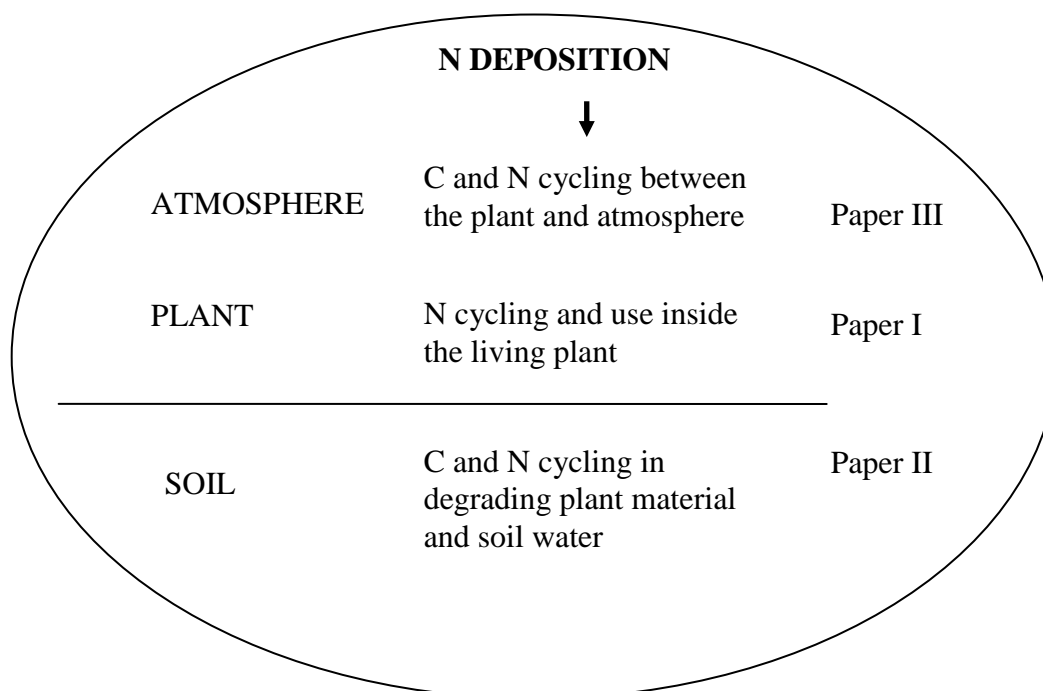


Figure 2.1. Approach for the study.

3. Material and methods

3.1. Study sites

The main study site at Whim bog was chosen because it has been used for studying the long term effects of nitrogen since 2002 and therefore provided an ideal site to look at the effects of N deposition on *Sphagnum* after long exposure time. To put the results from Whim into context and to see how representative they are of bogs of northern Britain in general, two additional sites were chosen. These sites were selected because they had similar vegetation to Whim and also because they both belong to the Environmental Change Network (ECN). This means that a plethora of measurements are carried out at the sites, including N deposition measurements.

3.1.1. Whim bog

Whim is an ombrotrophic bog situated c. 30 km south of Edinburgh (3°8'W, 55°46'N) (Fig. 3.1). It represents a transition between a lowland raised bog and a blanket bog. It is situated 280 meters above sea level and the annual rainfall is 900 mm, which is in the low end of the range of blanket bogs. The peat layer is 3-6 m deep and pH ranges from 3.27 to 3.91. The dominant vascular plants in the area are *Calluna vulgaris*, *Eriophorum vaginatum* and *Erica tetralix*. The field layer consists of several moss species (Table 3.1). The background N deposition is, approximately 8 kg ha⁻¹ y⁻¹; the N deposition was calculated at the site between 2002 and 2005 from weekly measured rainfall and nitrate (NO₃⁻) and ammonium (NH₄⁺) in the rainwater. The relatively small background deposition makes it a suitable site to study the effects of increased N deposition because the effects on control plots are relatively small. At the site less than half of the deposition comes as dry deposition of N; at the beginning of the experiment between July 2002 and June 2003 the wet deposition was 5.8 kg ha⁻¹ y⁻¹ and the dry deposition for the same period was 4.0 kg ha⁻¹ y⁻¹ (Sheppard *et al.* 2004; Leith *et al.* 2004). The dry deposition is more constant but the

wet deposition comes episodically with rainfall. (Sheppard *et al.* 2004)



Figure 3.1. Map showing the locations of the different study sites.

Table 3.1. Vegetation cover at Whim (derived from three 0.25 m² quadrats at each plot) according to Sheppard *et al.* (2004).

Species	Cover (%)
<i>Calluna vulgaris</i>	5-50
<i>Erica tetralix</i>	0-10
<i>Empetrum nigrum</i>	0-3
<i>Vaccinium myrtillus</i>	<1
<i>V. oxycoccus</i>	<1
<i>Eriophorum vaginatum</i>	0-30
<i>E. angustifolium</i>	<1
<i>Sphagnum capillifolium</i>	0-80
<i>S. papillosum</i>	0-20
<i>Pleurozium schreberi</i>	10-80
<i>Cladonia portentosa</i>	0-30
<i>Hypnum jutlandicum</i>	10-80

Whim bog is situated in southern Scotland where a more general anthropogenic influence has been significant for a long time. Therefore the bog is not pristine but rather a semi-natural ecosystem. The bog is surrounded by fields used for grazing and there are several farms and main roads nearby (Fig. 3.2). The western side of the bog from the study site is used for commercial peat harvesting which could possibly affect the drainage of the study site in the long term. The area harvested for peat has been drained so that 20 m from the plots there are ditches and a track. Transferring the peat from the harvesting site to the delivery vehicles can generate dust which may be deposited on the plots during dry periods as they use the track next to the study site. At the northern end of the bog there is an area that has been designated a SSSI (Site of Special Scientific Interest) for its peatland habitat interest (Gall 2000). The importance of the area is in the occurrence of nine *Sphagnum* species as well as *Calluna vulgaris*, many species of lichens, *Pinus sylvestris* and *Juniperus communis* (Gall 2000). The part of the bog that has been designated as SSSI has been found to be drying. This has been attributed to expansion of planted *Pinus sylvestris* and *Betula pubescens* (Gall 2000).



Figure 3.2. The approximate situation of the study site at Whim bog (small rectangle) and the boundaries of the SSSI site (large area).

3.1.2. Other study sites

Besides Whim two other study sites were included in the study (Figure 3.1). The study sites are situated at Moor House in northern England and the Cairngorms in northern Scotland. In all the sites vegetation is similar to Whim and *Sphagnum capillifolium* is abundant; they are classified as M19 (*Calluna vulgaris*- *Eriophorum vaginatum* blanket mire) in the National Vegetation Classification (Rodwell 1991).

These additional sites had different N depositions and also showed marked differences in environmental factors, e.g. rainfall, altitude and temperature (Table 3.2). These sites were used to evaluate how representative Whim is compared to other UK sites and compare it with less/more polluted sites. At these sites measurements of shoot extension, productivity, plant tissue nutrients and decomposition were carried out. For these measurements at each site three study plots of approximately 1 m² were chosen.

Table 3.2. Environmental variables of the study sites (Sheppard *et al.* 2004; Cundill *et al.* 2007; Rennie & Lane 1997).

Variable	Cairngorms	Whim	Moor House
Total N deposition (kg ha ⁻¹ y ⁻¹)	6	8	10
Oxidized:reduced N ratio	75:25	50:50	40:60
Rainfall (mm)	585	900	2055
Altitude (m.a.s.l.)	750	280	555
Mean annual temperature (°C)	5.2	7	6

Moor House

The Moor House study site is situated in the North Pennine uplands of England (2°45' W, 54°65'N). The area, which in total is 7500 ha, became an ECN site in 1992, and is one of the most-studied land areas in the UK, having been an IBP (International Biological Programme) site in the 1960s. Habitats of the site include exposed summits, extensive blanket peatlands, upland grasslands, pastures, hay meadows and deciduous woodland (Rennie & Lane 1997). The area is not heavily used although there is some recreational use as well as low-density sheep grazing (Cundill *et al.* 2007). The study site was situated in the area with blanket mire by the catchment of river Tees, which lies at the altitude of 555 m (Cundill *et al.* 2007). Blanket peat is the dominant soil type in the area. It was heavily eroded in the 1950s but has recovered since (Cundill *et al.* 2007). The peat depth is on average 2-3 m and the vegetation is dominated by *Calluna vulgaris*, *Eriophorum vaginatum* and *Sphagnum* spp. (pers. obs.). The mean annual temperature is 5.3 °C and the mean annual rainfall 1982 mm (Rennie & Lane 1997). Between 1994 and 2003 the average

annual N deposition was $10.3 \pm 1.3 \text{ kg ha}^{-1} \text{ y}^{-1}$, of which a little over half is deposited in reduced forms (Cundill *et al.* 2007; Rennie & Lane 1997).

The Moor house study site has been used in studying *Sphagnum* for a long time and lot of groundbreaking studies about the basic ecology of *Sphagnum* have been done there. Clymo (1973) studied the effect of water level, shading, pH and Ca^{2+} on the growth and productivity of different *Sphagnum* species. Clymo & Reddaway (1974) did cranked wire measurements at the site with *S. capillifolium* (subspecies *S. rubellum*) and found an annual length growth of up to 4 cm y^{-1} and productivity up to $135 \text{ g m}^{-2} \text{ y}^{-1}$. They also found that some wires were bent or went missing during the study and suspected grouse. Also in the current study some wires were lost at this site; in addition to grouse sheep might also have been responsible for removing the wires. Moor House has also been used for studying possible management solutions for semi-natural bogs (Rawes and Hobbs 1979) and the effects of burning and sheep grazing on the water table and depth and soil water quality of bogs (Worrall *et al.* 2007). Lately Moor House has also been used for studying the effects of N deposition on bogs, especially on soil water (Adamson 1998; Cundill *et al.* 2007).

Cairngorms

The Cairngormss study site is situated high in the Cairngorms, near Aviemore in Speyside ($3^{\circ} 49' \text{W}$, $57^{\circ} 06' \text{N}$) in the catchment of Allt a' Mharcaidh. The area, which is in total 1000 ha, has been intensively studied since the 1970s and it joined the ECN network in summer 1999. The habitats change according to the altitude from Caledonian pine woodland at 300 m up to arctic-alpine vegetation at 1100 m. The study site used in the present study is situated at the top of a hill at an altitude of 750 m. The vegetation comprises mainly *Calluna vulgaris*, *Eriophorum vaginatum*, *Cladonia portentosa* and *Sphagnum capillifolium* (pers. obs.). The mean annual temperature of the site is 5.5°C and the mean annual rainfall 585 mm. N deposition in the area is $6.2 \text{ kg ha}^{-1} \text{ y}^{-1}$, most being deposited in oxidized forms (Rennie & Lane 1997).

In the Cairngorms a lot of research has been done on the effects of tourism, mountaineering, skiing and hiking on the soil and vegetation (Nagy *et al.* 2002; Wood 1987). The Cairngorms has also been used for looking at N in stream waters (Chapman *et al.* 2001) and denitrification of deposited N in peat soils (Curtis *et al.* 2006).

3.2. Fertilization experiment at Whim study site

This project made use of an N manipulation experiment being carried out on Whim bog, where the effect of different N loads and forms (reduced and oxidized) are being studied. The experiment was started in 2002 and had been therefore running for four years before any measurements for this project were taken. The experimental area is in total 0.5 ha. At the study site there are 44 plots where the effects of wet N deposition are studied by adding active forms of nitrogen by spraying a solution.

N is added during rain events either as nitrate (NaNO_3) or ammonium (NH_4Cl) with doses of 8, 24 and 56 $\text{kg ha}^{-1} \text{y}^{-1}$ on top of the background deposition; in total the treated plots receive c. 16, 32 or 64 $\text{kg N ha}^{-1} \text{y}^{-1}$ (hereafter referred to as 8, 24 and 56 plots and NO is used to indicate nitrate and NH ammonium treated plots). The N is added with concentrations of 0.57, 1.71 and 4.0 mM, respectively. There are also plots with N load of 8 $\text{kg ha}^{-1} \text{y}^{-1}$ and 56 $\text{kg ha}^{-1} \text{y}^{-1}$ where phosphorus (P) and potassium (K) are added together with N (hereafter referred to as 8PK and 56PK plots). P and K are added as K_2HPO_4 at 1:14 ratio to N. The study area is divided into four blocks and the treatments are randomly assigned inside the blocks, each block containing one plot of every treatment (see Appendix I). In every block there is also a control plot which receives the same amount of rain water and therefore receives the background deposition of 8 $\text{kg ha}^{-1} \text{y}^{-1}$ of N. The plots are 12.5 m^2 , round shape and they are 3 m apart to avoid contamination from the next plot (Sheppard *et al.* 2004). In the study site there are movable board walks but due to the fact that they need two people for moving it was not possible to use them for most of the sampling. Therefore, walking around the study plots has probably caused some damage to the surrounding vegetation and water balance of the plots.

The N is added with rain water that is collected with a constructed 178 m² pitched surface and then fed into two 1250-l plastic water tanks. N is added to the rain water and then sprayed with sprinklers onto the study plots. The treatments add 10% of water to the rainfall volume. The spraying system only activates when it is raining, there is enough water in the tank and the wind speed is $< 5 \text{ m s}^{-1}$. The sprayed area exceeds the marked study plots (12.5 m²) with only 50% depositing to that study plot. The whole area was fenced in May 2007 to avoid rabbits causing damage on the plots. Rainfall, wind speed and direction, solar radiation, net radiation, air and soil temperature (10 and 20 cm below the surface) and water table level are constantly monitored in the area (Sheppard *et al.* 2004).

This novel way of fertilizing provides several advantages over other methods used in previous studies. Firstly, it mimics the N deposition in a more realistic way as the addition of N is coupled with rainfall as happens in the real world. The N is also added in small concentrations many times throughout the year instead of, for example, spraying only few times with large concentrations. The N loads used in the study are also realistic in the context of both UK and Europe.

For the experiments in the present study up to six microplots (c. 100 cm² or larger) of *Sphagnum capillifolium* from every study plot were chosen; in the control and 56 kg plots six microplots were chosen because one was used for the CO₂ measurements and in the other plots five microplots were chosen. The microplots were chosen where continuous *Sphagnum* cover was found in a patch of at least c. 100 cm². The number of microplots was restricted by the amount of *S. capillifolium* growing in some plots when less than five/six plots were chosen. In some plots with fewer patches of *Sphagnum* patches just outside the study plot, where the artificial deposition is still known to be the same as inside the plot, were chosen. All the microplots were colour-marked with plastic tags so that they could be identified.

3.3. Study species

In this study *Sphagnum capillifolium* is interpreted to include both *S. capillifolium* and *S. rubellum* following the British checklist (Hill *et al.* 2008). This is a British classification system where due to the difficulty of separating *S. capillifolium* and *S. rubellum* they are treated as subspecies. However, in the rest of the world they are classified as two different species. This is supported by a study by Cronberg (1997) where, using isozyme electrophoresis, he studied the genetical relationship between the two taxa and found that they indeed can be separated as species. A study by Hill (1976), on the contrary, suggested that they are one species and not even varieties of the same species.

My personal observation at the Whim study site was that around $\frac{3}{4}$ of the individuals could be attributed to subspecies *S. rubellum* and $\frac{1}{4}$ subspecies *S. capillifolium*. An earlier study looking at the taxonomy of these two taxa found *S. capillifolium* only from one out of eight British sites (the one site was situated at Moor House, England) confirming that most of the *Sphagnum* are in fact *S. rubellum* (Cronberg 1997). However, the author mentions having found *S. capillifolium* in earlier studies from other sites in Britain as well. *S. capillifolium* is found from more minerotrophic sites and *S. rubellum* from ombrotrophic to weakly minerotrophic sites. The separation of the taxa might be more difficult in Britain because in oceanic conditions *S. rubellum* occupies drier habitats than for example in Scandinavia, which has been noted by many field botanists (Hill 1976). The study by Cronberg (1997) also found that sometimes hybridization of the two species occurs, especially in intermediate habitats.

Combining the two taxa could have an impact when comparing the data with other studies. However, as the individuals of the two taxa are growing mixed at the study sites it would have been impossible to separate them. In addition, the results should represent *Sphagnum* species growing in ombrotrophic conditions in general and therefore should not have a large effect on the interpretation of the results.

3.4 Measurement of shoot extension, productivity and nutrient concentrations

The aims of the productivity experiment were to quantify the effects of different N loads and forms on the shoot extension, productivity and the amounts of nutrients in *Sphagnum capillifolium* and to assess the modifying effect of P and K when added with N.

Sphagnum productivity was studied using a variation of the cranked wire method (Clymo 1970), which is widely used for measuring the extension growth of *Sphagnum* mosses. In this method a metal wire is bent in the form of a vintage car starting handle and the part of the wire above the crank is measured to a known length. The wire is then inserted into *Sphagnum* mosses parallel to the stems of the mosses so that the crank is level with the capitula; the crank can then be used as a reference point against which to measure the extension growth of the mosses. To calculate the productivity, capitulum density and stem weight per unit of length are also needed. In the present study the part of the wire that was inserted into the *Sphagnum* carpet was bent in a cork screw form to help anchor the wires within the moss clumps (Fig. 3.3; see comparison of the method with other studies in Appendix II). Productivity of the mosses can then be calculated by multiplying the annual increment of the stem (cm), the density (individuals m⁻²) and the dry weight of the stem fraction (g cm⁻¹) (Clymo 1970).

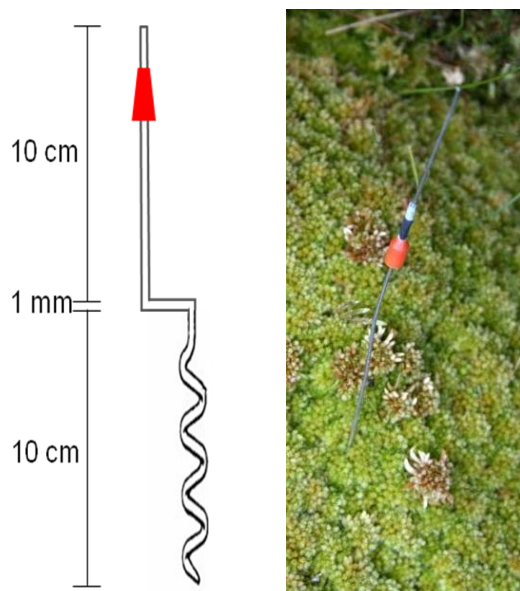


Figure 3.3. The design of the cranked wire and the upper part of a real cranked wire above the *Sphagnum* carpet in one of the study plots.

At Whim moss productivity was measured in all 44 study plots. Three cranked wires were inserted in five microplots of *S. capillifolium* (or less if the plot had less than five microplots); therefore in total all the study plots contained up to 15 cranked wires. All the wires were marked so that they could be identified. One microplot was used for measuring the density and the stem weight needed for calculating the productivity. In the other study sites five cranked wires were put into all three study plots, *i.e.* every study site had in total 15 wires. Also, all these study plots and wires were marked so that they could be identified. Measuring shoot extension and productivity in these sites followed the same procedure as at Whim.

At Whim the experiment was started in May 2007 and completed in November 2008 and at the other sites the experiments were started in June/July 2007 and finished in November 2008 (Moor House) and, due to less frequent visits because of snow and distance, in June 2009 (Cairngorms). Shoot extension was measured at Whim every three months (at the other sites every 4 to 6 months), starting at Whim in August 2007 and at the other sites in November 2007. The density was calculated from every plot in the beginning and in the end of the measurement period. The average of the two values was taken when calculating the productivity.

At the end of the experiment a sample of moss was taken from all study plots and 30 individuals were used to determine the productivity. The mosses were cut in two parts, capitulum (0-1 cm below capitulum) and stem fraction (1-3 cm below capitulum). The weight (in this method this means oven dry weight) of the stem part was used for calculating the productivity; the total weight of 30 stems was measured and this was averaged to represent the average weight of the stem in that study plot. The size of the capitulum can be affected by the increase in N and so can change during the experiment. To take this into account at Whim a sample of 30 capitula was weighed at the beginning and at the end of the experiment; at the other sites one measurement was done. A small change in the capitulum weight was found between the beginning and the end of the experiment at Whim. In the first measurement set at Whim the weights of 30 capitula varied between 0.065 and 0.184 and at the end of the experiment between 0.381 and 1.290. However, this was similar across the treatments (including control treatment) so this was assumed to be due to differences in the measurements or circumstances (for example time of year) between the two data sets. At Moor House the weights of 30 capitula were between 0.135 and 0.238 and at Cairngorms between 1.15 and 1.97.

At Whim at every study plot at the beginning, at the end and twice during the experiment moss was sampled for nutrient analysis. At the other sites samples were taken every time the site was visited. After collection, the samples were kept in the cold room (4°C). To avoid rinsing away some of the nutrients with water, the samples were thoroughly cleaned of adhering litter with tweezers. Capitula (0-1 cm), and at the beginning and in the end also stem parts (3-5 cm below capitulum), were removed for weighing. The samples were then oven-dried for at least 72 hours in 70 °C, ground with a ball-mill for 1 minute with the frequency of 20 shakes per second and analyzed for C and N with a Carlo Erba NA2500 total C/N analyser. In this method between 3 mg and 5 mg of sample material is weighed and the sample is introduced into the combustion tube via an auto-sampler. The combustion products are carried in the carrier gas, helium, through an oxidation catalyst and the excess oxygen is removed. After this the oxides of nitrogen are reduced to nitrogen which with the carbon dioxide and water passes through an absorbent filter, magnesium

perchlorate, for the water to be removed. The nitrogen and carbon dioxide are taken by the carrier gas to the chromatographic column and then to a thermal conductivity detector which generates electrical signals proportional to the concentrations of nitrogen and carbon dioxide present. The data are presented as a graph (retention time on x-axis and detector response on the y-axis) and the size of the peak represents the amount of analyte in the sample. The percentage of C and N is calculated by comparing the size of the peak of the sample to the peaks of the standards. The first set of measurements were done at a different laboratory otherwise using the same procedure except that the sample was heated at 105°C for 1h after being ground. At the first two measurement times other nutrients were also analysed from the capitulum parts. For these the analysis was done with Bran+Lubbe Auto Analyser (P) and with Solaar M series Unicam Atomic Absorption Instrument (K, Ca and Mg).

3.5. Evaluating visual damage

The aim of this experiment was to relate the visual damage that could be seen in the study plots to the possible measured changes in *Sphagnum* health (changes in shoot extension and productivity).

This experiment was only carried out at Whim study site. In May 2008 a score between 1 and 5 was assigned to all the microplots according to how healthy looking *Sphagnum* in the plot was (Fig. 3.4). The plot scored five when *Sphagnum* looked very healthy and moist and only one when *Sphagnum* looked very unhealthy, there was lot of algae, it was very dry, the hummock was falling apart or a lot of other visible damage could be seen. The other three classes were scaled between these two, for example in class four some damage was seen, in class three more and so on leading to class one where *Sphagnum* looked very unhealthy.

Weather can have a large effect on assessing visual health as dry *Sphagnum* looks much more damaged than when wet (for example the colour changes from green to whitish). This problem was avoided by assessing all the plots during one day.

However, this could have an effect if this method was used in assessing several sites in different weather conditions.



Figure 3.4. Examples of *Sphagnum* stands from different score classes used in evaluating the visual damage. Score 1 was given to *Sphagnum* stands that looked very unhealthy and score 5 to visually healthy looking ones.

3.6. Measurements of decomposition rate using litter bags

The aim of the decomposition experiment was to quantify the effects of different N loads and forms on the decomposition rate of *S. capillifolium*, and to assess the modifying effect of P and K when added with N.

At Whim the material for the decomposition experiment was collected in the beginning of March 2007 and at the other sites in June/July 2007. Approximately 250-300 individuals of *S. capillifolium* were collected from every study plot from one of the microplots. To avoid rinsing away some of the nutrients with water the samples were thoroughly cleaned with tweezers and stem parts (3-8 cm below top of the capitulum) were cut. The part of the stem used for the decomposition experiment

was chosen based on the colour and texture of the stem. At this depth (3 cm below the capitulum) the moss changed from green to fawn indicating that it is not living tissue anymore and therefore in natural conditions would be decomposing. The stem parts were then divided into five portions. One portion was oven-dried for 48 hours in 70 °C and used for chemical analysis following the same procedure as described with capitulum nutrients above. The remaining four portions were air-dried for at least 76 hours and then put into litterbags. The samples were air-dried to avoid changes in the chemical composition; a test was done previously in February 2007 to find out how long *Sphagnum* takes to air-dry, i.e. after what time the weight at room temperature does not change anymore. The litterbags were made of 0.71 mm mesh polypropylene mesh (Fig. 3.5; see comparison of the method with other studies in Appendix III). The material was chosen because polypropylene has a good resistance to acidic conditions and the mesh size was chosen because this size is small enough to prevent the material falling out and for excluding macrofauna. One portion of about 0.5 g (0.3-0.9 g) of air-dried sample was put into each litterbag.



Figure 3.5. Litterbags used for measuring the decomposition rate before incubating in the field.

The litter bags were buried at Whim in April 2007 and in the other sites in July/August 2007 (Fig. 3.6). Four litter bags were incubated in every study plot. The litter bags were placed 5-10 cm below the surface i.e. approximately the same depth where the samples had been taken. At Whim one litter bag was harvested every 6 months (October 2007, April 2008, October 2008 and April 2009), in other sites one

bag was taken up every time that the site was visited (every 4 to 6 months). Mass loss from the samples was established by air-drying until dry and weighing the sample. The sample was then oven-dried, ground and analyzed for C and N. The analysis followed the same procedure as previously described.



Figure 3.6. Litterbags in the field were buried 5-10 cm below surface and a wire was attached to each bag to prevent losing them. The wires were colour coded to be able to identify each bag.

3.7. Water samples from rhizon filters

The aim was to quantify the amount of N that leaches to the pore water, to see how much C is lost as DOC and to see how these relate to the decomposition rates in different treatments.

The water samples were collected by using rhizon soil moisture samplers (Eijelkamp Agrisearch Equipment 19.21, The Netherlands). Rhizon samplers consist of a 10 cm porous polymer tube with pore size of 0.45 μm (the size of DOC) that is connected to a 10 cm PVC tube. At Whim, rhizon samplers were placed alongside the litter bags to collect water samples from the moss layer at the same depth where the litter bags were incubated, *i.e.* 5-10 cm below the moss surface. They mimic the behaviour of plant roots and collect water and nutrients from the flowing water like a root would, *i.e.* under suction. This collected water is then sucked into a syringe attached to the rhizon filter where the suction is maintained by jamming the syringe open. Water was collected from the samplers if possible twice a month, but at least once every month, during year 2008. Because the amount of water in the syringe depends on the amount of rain and the moisture level of *Sphagnum*, it was not always possible to get a sample from every plot or for all the types of analysis, so the number of samples was different for every measurement time. After the samples were taken they were kept cool during transportation.

When in the laboratory, the sample was divided in two and the sub sample destined for measuring DOC was frozen immediately to await analysis. The other half was further divided into two and one quarter was used for measuring pH (Mettler Toledo MP220 pH meter) after which it was discarded and the other quarter was filtered and then frozen to wait for the analysis of total N, NH_4 and NO_3/NO_2 . Later on DOC was measured using DC-80 Total Organic Carbon (TOC) analyser (Rosemount-Dohrmann) using a sub sample of 200 μl . Total N, NH_4 and NO_3/NO_2 were measured with a continuous flow analyser (San ++ Continuous flow analyser, Skalar analytical BV, the Netherlands).

3.8. CO₂ flux measurements using static chambers

The aim of the CO₂ experiment was to quantify the rates of CO₂ fluxes and assess if adding P and K with N changes these fluxes. A secondary aim was to see how well the data from the cranked wire and litter bag methods relate to measured CO₂ fluxes.

Only control and 56 kg treatments were used for this experiment due to the time consuming nature of chamber flux measurements. From all the control plots and all 56 and 56PK plots stands with pure *S. capillifolium* were selected (in total 20 plots). The stands were relatively small (diameter c. 10 cm) for two reasons: at the site *S. capillifolium* does not, in general, form large uniform areas and especially in the high N plots the amount of *Sphagnum* left after 6 years of treatment prior to the study was not large. Collars were made out of drain pipe and they were approximately 15 cm deep with a 10 cm radius. In July 2007 the collars were inserted into a subjectively chosen spot in all of the 20 plots used for this study (Fig. 3.7). These locations were chosen to be (i) representative of the *S. capillifolium* in the plot in general and (ii) accessible from outside the plot.



Figure 3.7. The collar used for the CO₂ measurements inserted into the *Sphagnum* carpet.

Measurements were started 3 months after inserting the collars, so that the stands had time to recover from the stress induced. The measurements were made between October 2007 and November 2008. The amount of measurements per month was dictated by outside temperature and availability of equipment, but where possible, measurements were undertaken several times a month. The measurements were carried out in different conditions and different times of day to get as wide a range of weather conditions as possible. However, rainy conditions precluded measurement,

due to the likelihood of equipment failure. One set of diurnal measurements was made in July 2008 when the CO₂ fluxes were measured every two hours for a 24 hour period to investigate the diurnal patterns of net ecosystem productivity (NEP) and total ecosystem respiration (ER). Only half of the plots used for this experiment were used for the diurnal measurements (10 plots) and they were subjectively chosen.

Instantaneous net ecosystem CO₂ exchange was measured with a transparent chamber (height 11.2 cm, volume 775 cm³) and a portable infrared gas analyser (EGM-3 and EGM-4, PP systems, UK) connected to make a closed air circulation. The chamber had a fan to circulate the air inside it and it was attached to the collar with a rubber seal to make it airtight. Measurements lasted up to two minutes or until the CO₂ concentration had changed by more than 50 ppm. One or two measurements were undertaken in different photosynthetically active radiation (PAR) levels so that the PAR level stayed constant during one measurement. After this the chamber was covered with dark cloth to measure ecosystem dark respiration. The chamber was removed from the collar between the measurements to restore the ambient gas concentration. The EGM recorded the CO₂ concentration, air temperature and PAR every four seconds during the measurement. Soil temperature from 5 cm and 10 cm below the moss surface and water table were measured at the same times as the gas exchange measurements were done.

Hourly fluxes of NEP, ER and gross photosynthesis (P_G) were calculated from the linear change in gas concentration in the chamber as a function of time. Only data where there was a linear increase in concentration passed the quality test and the non-linear measurements were discarded. From the measured NEP and ER fluxes an estimation of gross photosynthesis (P_G) was calculated as the difference between the two. The data are presented so that negative fluxes represent loss of CO₂ from the vegetation and positive fluxes represent gains of CO₂ for the vegetation. For every month in 2008 when measurements were done (all except January, September and December) a monthly average for NEP, ER and P_G was calculated. In this, the hourly flux was multiplied first by 24 and then by the number of days to get the monthly

balance. In the calculation it was assumed that there was 12 hours of daylight (NEP flux) and 12 hours night (ER flux) all year round. It was also tested to see if changing the hours at summer time (three months of four hours of dark) and winter time (three months of four hours of light) would change the results but because this did not affect the results much, it was decided to use 12 hours of light and dark year round. Because no measurements were done in January, September and December, a value for these months was calculated assuming linear change in the fluxes. The cumulative annual CO₂ balance for each treatment was calculated by adding monthly NEP (daytime) and ER (night time) values together.

3.9. Other measurements

3.9.1. Water table

Water table is very important for *Sphagnum* because the plants receive all their water from the surrounding environment. The aim of measuring water table was to make sure that possible differences between the different treatments can be taken into account when interpreting the results. In the control and “N only” plots there were existing dip wells already before the present study started, but for the PK plots the dip wells were inserted in the beginning of these experiments. Dip well heights were measured normally twice a month but sometimes more often (occasionally only once due to lack of time). There was one dip well per plot and the difference to the CO₂ collars was estimated to calculate a water table for them (the water table at CO₂ collars is presented in Paper III and the water table for the whole plot in Chapter 7).

3.9.2. Vegetation description

Aim of the vegetation description was to give background information on the conditions of *Sphagnum* in the different microplots, for example the amount of shading. Prior to the start of the present study in spring 2007 all the microplots were

described. The survey included the plants that were growing together with *S. capillifolium* in the microplots and those surrounding *Sphagnum*. ‘Surrounding’ here was a subjective decision made by the measurer; as a general rule plants that grew in the close proximity or close enough to cast shade on *Sphagnum* were described. The descriptions were by a single observer to minimise observer error. The species present and a two-point indication of quantity (abundant, uncommon) were recorded. A comprehensive list of surrounding plants is included in the Appendix IV.

Change in percentage cover for *S. capillifolium* and other dominant species was assessed initially at the start of the experiment in May 2002 and then in 2004, 2007 and 2009 from both the entire study plots (12.5 m²) and permanent quadrats (3×0.25 m² in each study plot).

3.9.3. *Sphagnum* pH

The aim of measuring *Sphagnum* pH was to see if the measured changes in the pH of pore water are affecting *Sphagnum*. *Sphagnum* pH was measured from two sets of *Sphagnum* samples collected in May and June 2008. From every plot a sample was collected from the same microplot used for the productivity experiment. After collection samples were transported to laboratory and kept in the fridge until processed. Within a few weeks samples were cleaned with tweezers to remove any litter or other plant species. After this deionised water was added to the sample in 1:8 ratio (8 ml of water per g of *Sphagnum*) and they were ground in a ball mill. After grinding the pH of the solution was measured with a pH meter (Mettler Toledo MP220 pH meter). The samples from both measurement times were very similar and an average was calculated for every plot from the May and June measurements.

3.9.4. *Sphagnum* water content

The aim of measuring *Sphagnum* fw/dw was to determine whether adding N decreases the capacity of *Sphagnum* to hold water. Twice a month at the same time

when the dip wells were measured and the water samples from the rhizon samplers taken a small sample (less than 10 individuals) of *Sphagnum* was taken from one microplot in every study plot (the one used for productivity and litter bags). These samples were transported in plastic bags to the laboratory as quickly as possible and they were kept in the fridge until handled. As soon as possible after returning to the laboratory between 1 and 6 individuals from every sample was/were chosen, the first three cm cut off and weighed instantly to get the fresh weight. After this the individuals were dried in the oven at 70°C at least for 72 hours after which they were weighed again to get the dry weight. A fresh weight/dry weight ratio was calculated for every plot using the total weight of all the individuals. This fw/dw ratio was then plotted against the measured water table in the microplot to see if there was a relationship between the water table and the moisture level of *Sphagnum*.

3.9.5. N₂O and CH₄ measurements

The aim of measuring the other two important greenhouse gases was to obtain an estimate of the scale of these fluxes compared to CO₂, and to elucidate the total C and N balance. Due to lack of time the measurements were only carried out once and therefore the results cannot be treated as representative of long-term means. Both of these fluxes are dependant on weather and season, especially for methane wet conditions with high water table can increase the flux considerably. However, the measurements were done on a rainy day which should remove part of the problem. In addition, at Whim the water table is never very high, especially not in the hummocks where the present study was carried out, which should make fluctuations in these fluxes smaller. Supporting this all the measured fluxes of NH₄ at Whim have been less than 30 mg m⁻² h⁻¹ and no significant effect of N additions have been found (Sheppard *et al.* 2008).

In August 2008 one set of N₂O and CH₄ measurement was made in the control, 56 and 56PK plots (same plots that were used for the CO₂ flux measurements). The measurements were made on two different days because it was not possible to carry out all measurements in one day with only one chamber. To do the measurements the

chamber used for measuring CO₂ was covered in foil to keep it dark, the inside temperature stable and to prevent photosynthesis during the measurement. Three-way valves were inserted into the plastic tubes leaving from the chamber that normally are connected to the portable infrared gas analyser (EGM) normally used for CO₂ measurements. The other valve was kept closed during the measurement and a syringe was attached to the other one. An air sample was drawn into the syringe from the chamber after 5, 10, 15 and 20 minutes from closing the chamber. Only about 50 ml of air was taken into every syringe to avoid a low pressure developing in the chamber, thus sucking more gases from the soil. At the same time with the air sample, the temperature inside the chamber, the air temperature and the depth of the water table were recorded. The samples were kept cool while continuing the measurements and transporting them into the laboratory.

In the laboratory the samples were analysed with a gas chromatograph (HP5890 Series II gas chromatograph with electron capture (ECD) and flame ionisation detectors (FID) for N₂O and CH₄, respectively) the same or the following day. The working principle of a gas chromatograph is explained in chapter 3.2. Based on the concentration results from the gas chromatograph, CH₄ and N₂O fluxes were then calculated as a linear change in gas concentration in the chamber as a function of time.

3.9.6. N and C balance

Using all the data it was possible to calculate a C and N balance for the control, 56 and 56PK plots for the year 2008. For the C balance an average monthly CO₂ balance and an average monthly DOC discharge were added to estimate the amount of C released monthly.

Monthly discharge of DOC was calculated using the monthly rainfall at Whim and monthly evapotranspiration at a very nearby Auchencorth moss to calculate the amount of water in litres running through one square metre of *Sphagnum* and this was used with average monthly DOC (mg/l) measured in the soil pore water and

collected with rhizon samplers to calculate C loss as DOC. No DOC measurements were carried out in December 2008 so it was assumed to be the same as in November (based on the data this is probably an overestimation of DOC released). This method assumes that all DOC that was measured is moving vertically and there is no lateral water movement bringing DOC to the plots from surrounding areas. This assumption is probably safe in the hummocks at Whim which are fairly dry and above the general water table of the site. The calculation of the monthly average CO₂ flux is explained in Chapter 3.6. The single measurement of CH₄ flux was also added for every month. The C balance represents losses of C also from sources other than *Sphagnum* as in ER and DOC loss it is not possible to separate the effects of surrounding vascular plants and their roots.

For the N balance the single measurement of N₂O flux was added to the average monthly total N in pore water. N₂O flux was only measured once but because it was very small, the same flux was used for every month assuming that changes in it would be small during the year. Total N in the pore water was measured in other months except March, May, July, November and December. For these months discharge of total N in the pore water was estimated based on the other months assuming linear change. After this the monthly discharge of N in pore water was calculated in a similar way to DOC.

4. Paper I “How do long-term, nitrogen additions as ammonium or nitrate affect growth, productivity and nitrogen concentrations in *Sphagnum capillifolium* (Ehrh.) Hedw.”

Sanna K. Kivimäki, Sally Johnson, Ian D. Leith and Lucy J. Sheppard

Summary

This study has used a unique ‘real world’ treatment regime to examine the effects of N form, ammonium and nitrate, and N load, 8, 24 and 56 kg N ha⁻¹ y⁻¹ above the ambient, with and without PK additions, on shoot extension, productivity and the tissue nutrient concentrations (C, N, Ca, Mg, P and K) in the hummock forming *Sphagnum capillifolium*. Visual assessments of health were also made to see if N treatment cause specific changes in the appearance of *S. capillifolium* that could be used for monitoring. Whim bog, in southern Scotland is in an area of relatively low background N (and S) deposition (8 kg N or S ha⁻¹ y⁻¹) and N deposition has been manipulated since 2002. No effect of N form was found and N addition caused negative effects although the dose did not have a significant effect; same was true for both shoot extension and productivity. Relieving the PK limitation for this species caused significant changes in the N response. Adding N decreased shoot extension and productivity of *S. capillifolium* but simultaneous P and K additions alleviated the effects of N. Both capitulum and stem %N increased with increasing N dose: in capitula concentrations of > 20 mg g⁻¹ were measured. Both N:P and N:K ratios were initially high and increased with N additions. Visual assessments correlated well with tissue chemistry and productivity; decline in health was associated with high %N and reduced productivity. This study confirms the results obtained from short-term experiments where N additions have been less well coupled to real world conditions; increased N deposition decreased the vitality of *Sphagnum*. No adaptation to increased N was found after 6 years of fertilization. While adding PK alleviated N effects it also caused changes in the species composition, increasing competition from hypnaceous mosses.

Introduction

Bogs are naturally nutrient poor ecosystems and because of this, bog vegetation, especially *Sphagnum* mosses, are well adapted to low nutrient availability (Chapin *et al.* 2004). In pristine conditions *Sphagnum* mosses are able to take up most of the atmospherically deposited N making it unavailable for vascular plants (Aerts *et al.* 1992; Lamers *et al.* 2000) therefore favouring their own growth (Van Breemen, 1995). *Sphagnum* mosses also grow indefinitely from the capitulum and decompose very slowly; these qualities make them the main peat formers in ombrotrophic bogs (Clymo & Hayward 1982; Van Breemen 1995). It has been estimated that about 455 Gt of C has been stored in peat in boreal peatlands since the last glacial period (Post *et al.* 1982; Gorham 1991) and that these ecosystems currently sequester 0.07Gt y^{-1} of C annually (Clymo *et al.* 1998), thus *Sphagnum* plays a crucial role in the global C cycle.

In the last few decades N deposition has been increasing in many areas of the northern hemisphere due to anthropogenic activities; industrialization, intensive agriculture and fossil fuel burning have led to three to five fold increases in N emissions in the last century (Reay *et al.* 2008). In the UK, the total annual deposition of N averages to $\sim 17\text{ kg ha}^{-1}\text{ y}^{-1}$ and in certain areas in Europe deposition can reach $60\text{ kg ha}^{-1}\text{ y}^{-1}$ (Lamers *et al.* 2000; NEG-TAP 2001). These levels are considerably higher than the critical load (CL), which for bogs has been estimated to be $5\text{-}10\text{ kg ha}^{-1}\text{ y}^{-1}$ (Bobbink & Roelofs 1995; Bragazza *et al.* 2004). In some studies increasing N availability has led to increased productivity of *Sphagnum* mosses, although such positive responses have been restricted either to short term additions, small doses or areas with low background N deposition (Rocheffort *et al.* 1990; Aerts *et al.* 1992; Vitt *et al.* 2003).

In the long term increased N deposition has led to failure of the *Sphagnum* filter and more N leaching to the rhizosphere; this can lead to changes in the species community increasing the amount of grasses and reducing the amount of *Sphagnum* (Lamers *et al.* 2000; Berendse *et al.* 2001; Limpens *et al.* 2003a). The threshold for

changing the direction of response, from positive to negative, appears to happen when the deposition reaches $\sim 18 \text{ kg ha}^{-1} \text{ y}^{-1}$ and the *Sphagnum* N concentration $\sim 20 \text{ mg g}^{-1}$ (Lamers *et al.* 2000; Berendse *et al.* 2001; Limpens *et al.* 2003a). Supporting observations have been found for example in The Netherlands, where N deposition of $40 \text{ kg ha}^{-1} \text{ y}^{-1}$ depressed shoot extension and net primary production of several *Sphagnum* species (Limpens *et al.* 2004). In another study by Limpens *et al.* (2003a) the cumulative shoot extension of *Sphagnum* over two growing seasons was reduced from 6 to 4 cm when N dose was increased from $40 \text{ kg ha}^{-1} \text{ y}^{-1}$ to $80 \text{ kg ha}^{-1} \text{ y}^{-1}$. However, increasing the N dose from 0 to $40 \text{ kg ha}^{-1} \text{ y}^{-1}$ had no effect which suggests a higher threshold than the $\sim 18 \text{ kg ha}^{-1} \text{ y}^{-1}$ proposed threshold for negative change. Some studies have also shown that in the areas with a longer history of high N deposition the growth decreases with increasing N deposition whereas no effect is found in less N polluted areas or that the initial positive effects eventually turn into negative effects with time (Rocheffort *et al.* 1990; Berendse *et al.* 2001). These observations suggest that N effects on *Sphagnum* may be cumulative. It is also possible that *Sphagnum* growing in very N clean areas are particularly N sensitive and have lower N deposition thresholds than *Sphagnum* growing in dirtier areas. Vitt *et al.* (2003) found in a study in Canada that negative impacts of N on net primary production of *S. fuscum* started to appear when the N deposition reached only 14.8-15.7 $\text{kg ha}^{-1} \text{ y}^{-1}$ which is much closer to the critical load estimation for European bogs.

It is also apparent that N responses are species specific in *Sphagnum* with hummock forming species found to be more sensitive to increased N than species growing in hollows (Gunnarsson & Rydin 2000). This may be due to their natural habitat that exposes them to more deposition and/or higher N concentrations (less water to dilute N) or because they have a higher uptake rate of N and especially ammonium (Jauhiainen *et al.* 1998). Carfrae *et al.* (2007) found that increased N deposition reduced branching of hummock forming *Sphagnum capillifolium* and Gunnarsson & Rydin (2000) suggested, based on their observations that hummocks might collapse under high N deposition due to changes in the structure of *Sphagnum*.

Besides N, phosphorous (P) and potassium (K) also have an important role in peatland ecology. Some peatlands can be P- or K- limited or co-limited by both nutrients and the increased N deposition can shift the limiting nutrient from N to P and/or K (Aerts *et al.* 1992; Hoosbeek *et al.* 2002; Bragazza *et al.* 2004; Limpens *et al.* 2004). The N/P and N/K ratios can be used as indicators for the limiting nutrient in the ecosystem (Koerselman & Meuleman 1996; Hoosbeek *et al.* 2002).

Koerselman & Meuleman (1996) suggested for wetland species that if the N/P ratio is < 14 the ecosystem is N-limited, if it is between 14 and 16 it is co-limited by the two nutrients and if it exceeds 16, the ecosystem is P-limited. Hoosbeek *et al.* (2002) suggested similar relationship for N and K so that when N/K-ratio is below 1.2 it indicates N-limitation, ratio $1.2 < N/K < 1.4$ indicates N and K co-limitation and ratio > 1.4 indicates K-limitation. However, Bragazza *et al.* 2004 suggested considerably higher ratios of N:P ratio of ~ 30 and N:K ratio of 3.3-3.4 as the limits for changing from N limitation to P or K-limitation.

Although P and K are very important in peatland ecosystems, there has been less emphasis placed on them, but some studies have looked at the effects of P and K as well as N. Limpens *et al.* (2004) found that adding P ($3 \text{ kg ha}^{-1} \text{ y}^{-1}$) stimulated the shoot extension and productivity of different *Sphagnum* species and thus suggested that the studied bogs had become P-limited. In a study undertaken in the second year of treatments at the Whim study site, Carfrae *et al.* (2007) found that adding P and K with N reduced the foliar N content and promoted the growth of *S. capillifolium* (measured as the amount of photosynthesising material). On the other hand, Bubier *et al.* (2007) found in a 5-year study a decline in *S. capillifolium* and *S. magellanicum* growth when only P ($50 \text{ kg ha}^{-1} \text{ y}^{-1}$) and K ($63 \text{ kg ha}^{-1} \text{ y}^{-1}$) were added or they were added together with increased N. Because they found a stronger negative effect of PK and NPK additions than 'N only' additions, they suggested that PK might be more 'toxic' than N. However, the amount of P and K added in the Bubier *et al.* (2007) study was extremely high and will therefore undoubtedly lead to toxic effect. Besides often becoming the limiting nutrient in high N deposition areas, P and its availability can influence the impact of N deposition by enhancing *Sphagnum*'s

capacity to assimilate N (Limpens *et al.* 2004). Thus, the role of these three nutrients in determining the growth of *Sphagnum* mosses is far from clear.

Most of the studies referenced above have been relatively short-term but have underlined the importance of long-term measurements (Gunnarsson & Rydin 2000; Bubier *et al.* 2007). In addition, previous N addition experiments have not simulated wet N deposition realistically, as N has been added only a few times per growing season, which might have influenced the responses of the studied species compared to real life N deposition that comes year round, in small doses and low concentrations. In this study we have addressed these shortfalls by using wet treatment applications that are coupled to rainfall and extended the investigations to take account of the form in which the N is applied.

The aims of this study were to (i) quantify the effects of different N loads and forms after five years of enhanced N deposition on shoot extension, productivity and nutrient concentrations in *Sphagnum capillifolium* (Ehrh.) Hedw., (ii) assess if the oxidized and reduced N forms have different effects, (iii) assess how PK modifies the N response and (iv) determine if visual changes can be used as an indicator for the changes caused by increased N, so offering a cheap robust monitoring tool for the Conservation Agencies.

We hypothesized that after a long exposure time for increased N deposition only the lowest N dose ($8 \text{ kg ha}^{-1} \text{ y}^{-1}$) would still increase the growth and productivity and the higher doses would have a detrimental effect and that they would decrease productivity. Adding PK with N was expected to increase the P/K ratio and remove the PK limitation; this was hypothesized to increase the growth and productivity compared to 'N only' treatments.

Material and Methods

Study sites

The study was carried out at three different sites in the northern UK. The main study site was Whim bog, an ombrotrophic bog situated c. 30 km south of Edinburgh, Scotland (3°16'W, 55°46'N). To be able to relate the results from the Whim experimental site to other northern bogs two additional sites were identified. Both additional sites belong to the Environmental Change Network (ECN) (Rennie & Lane 1997) which holds a lot of background information concerning meteorological and chemical drivers. The sites were in northern England, Moor House and in the Cairngorms in Scotland (Table 4.1). The vegetation at all three sites consists mainly of *Calluna vulgaris*, *Eriophorum vaginatum*, *Erica tetralix*, *Cladonia portentosa* and *Sphagnum capillifolium*. The main vascular plant is *Calluna vulgaris* covering up to 50% of the area in all the study sites and the height of the vascular plants is approximately 30-40 cm. At each site the *Sphagnum* plots were chosen from as open places as possible so that they were shaded by the vascular plants as little as possible. In this study *S. capillifolium* is interpreted to include both subspecies *capillifolium* and *rubellum*.

Table 4.1. Environmental conditions at the study sites.

Variable	Cairngorms	Whim	Moor House
Total N deposition ($\text{kg ha}^{-1} \text{y}^{-1}$)	6	8	10
Oxidized:reduced N ratio	75:25	50:50	40:60
Rainfall (mm)	585	900	2055
Altitude (m.a.s.l.)	750	280	555
Mean annual temperature (°C)	5.2	7	6

Whim bog represents a transition between a lowland raised bog and a blanket bog. It is in the dry end of the occurrence of raised bogs. However, the weather during this experiment was very wet in Scotland and the rainfall was above average. In 2007 the total rainfall was 1235 mm and in 2008 1400 mm. The background N deposition is relatively small, approximately $8 \text{ kg ha}^{-1} \text{y}^{-1}$ increasing proportionally in relation to rainfall above 900 mm (Sheppard *et al.* 2004). As both of the study years were wet,

the deposition is expected to be slightly higher. At Whim bog the N manipulation study has been running since 2002, enabling longer term effects to be evaluated. In the experiment N is added to 44 plots (12.5 m² round plots 3 m apart from each other) in two N forms, nitrate (NaNO₃) and ammonium (NH₄Cl), in doses of 8, 24 and 56 kg ha⁻¹ y⁻¹ on top of the background deposition (hereafter referred to as NO/NH 8/24/56 plots). There are also plots with N loads of 8 kg ha⁻¹ y⁻¹ and 56 kg ha⁻¹ y⁻¹ where P and K are added together with N (hereafter referred to as NO8PK, NO56PK, NH8PK or NH56PK). P and K are added as K₂HPO₄ with a P:N ratio of 1:14. There are four plots per treatment, including control treatments that only receive additional rainwater. N is added to the rain water that has been collected at the study site and distributed from a central rotating disc at the centre of every study plot. The spraying only activates during low wind speeds to assure even distribution of water. The treatments increase precipitation on the plots by ~10% (for details of the experimental design see Sheppard *et al.*, 2004). Rainfall, wind speed and direction, solar radiation, net radiation, air and soil temperature (10 and 20 cm below the surface) and water table level are constantly monitored. In the 'N only' plots dip wells were installed in 2006 but in the NPK plots the dip wells were inserted in autumn 2007. In the 'N only' plots the dipwells are situated on the lawns and in the NPK plots in the hummocks. Because of the nature of hummocks at this site often standing up to 50 cm above the lowest point in the plots and because of the large size of the plots the dipwell values can only provide an indication of water movement up and down, not a measure of the actual water table in the microplots.

The Moor House study site is situated in the North Pennine uplands of England (2°45' W, 54°65'N). The area is not heavily used, although there is some recreational use as well as low-density sheep grazing (Cundill *et al.* 2007). The study area is situated in the area with blanket mire in the catchment of the river Tees and was heavily eroded in the 1950's but has recovered since (Cundill *et al.* 2007). The peat depth averages 2-3 m. The Cairngorms study site is situated high in the Cairngorms, near Aviemore in Speyside (3° 49'W, 57° 06'N) in the catchment of Allt a' Mharcaidh. The area, which totals 1000 ha, has been intensively studied since the 1970's and became part of the ECN network in the summer of 1999.

At Whim up to five microplots of *Sphagnum capillifolium* were chosen in every study plot. The microplots were chosen where continuous *Sphagnum* cover was found in an area of at least 100 cm². However some plots, especially those belonging to the high N treatments, had insufficient areas of *S. capillifolium*. In these plots, areas just outside the study plot, where the artificial deposition is still approximately the same as inside the plot, were used. At the other two sites three study plots were chosen from similar vegetation to Whim, with full cover of *S. capillifolium* present and five microplots of *S. capillifolium* were identified within each study plot.

Field and laboratory measurements

Sphagnum productivity was studied using a variation of the cranked wire method (Clymo 1970), which is widely used for measuring the productivity of *Sphagnum* mosses. In this method a metal wire is bent in the form of a car starting handle and the part of the wire above the crank is a known length. The wire is then inserted into the *Sphagnum* hummock parallel to the stems of the individual capitula so that the crank is at their level; the crank can then be used as a reference point to measure the shoot extension of the mosses. The part of the wire that was inserted into the *Sphagnum* carpet was bent into a cork screw form to anchor the wires into the moss stems. To calculate the productivity, capitulum density and stem weight are also needed. Productivity was calculated by multiplying the annual growth of the stem (cm y⁻¹), the density (individuals m⁻²) and the dry weight of the stem fraction (g cm⁻¹) (Clymo 1970).

At Whim in May 2007 three cranked wires were inserted into each microplot of *S. capillifolium*; therefore in total most of the study plots had 15 cranked wires and all the plots had at least nine. Shoot extension (the height of the capitula compared to the crank) was measured every three months, starting in August 2007 and continuing until November 2008. The *Sphagnum* carpet can compress slightly, especially under snow, during the measurements. Compression because of decomposition is assumed to be minimal in the first 10 cm of *Sphagnum* where the wire was attached. However,

this can be assumed to be similar in all the study plots at one site and therefore should not affect comparison between the treatments but could cause some error in between site comparisons due to larger amount of snow at the other two sites. To avoid biasing the results by having two summers but only one winter, the productivity calculations are based on the first 12 months. The density was estimated from one microplot in every study plot in the beginning and at the end of the measurement period. The density (an average of the two measurements) varied between 6121 and 28083 individuals m^{-2} in the different study plots. The average of the two values was used when calculating the productivity. Only using one microplot for counting density was due to time constraint and it could cause an error to the results because all five microplots were used for measuring shoot extension. However, the density is not expected to vary greatly within one study plot and there are four replicates of every treatment which should provide a realistic estimate for the treatment.

At the end of the experiment a sample of moss was taken from one microplot per plot and 30 individuals from this sample were used for determining the stem weight (where stem includes all the branches and leaves attached to it). The mosses were cut in two parts, capitulum (0-1 cm below the apex of the main stem) and stem fraction (1-3 cm below the top). The dry weight of the stem part was used for calculating the productivity; the total dry weight of 30 stems was measured and this was averaged to represent the average weight of the stems in that study plot. The size of the capitulum can be affected by the increase in N and so can change during the experiment. To take account of this a sample of 30 capitula was weighed in the beginning, in the middle and at the end of the experiment. A small change in the capitulum weight was found between the beginning and the end of the experiment but this was similar across the treatments (including control treatment) so this was assumed to be due to differences in the measurements or circumstances between the data sets rather than an N treatment effect.

At the other two sites five cranked wires were inserted into every microplot, i.e. both sites had 15 cranked wires. At Moor House the study was started in June 2007 and

the wires were measured every 4-5 months until November 2008. At Cairngorms the study was started in July 2007 and the wires were measured three times over two years. The density of individuals from all the study plots at both sites was estimated once. A sample for the stem weight was taken at last visit and the same procedures as used at Whim were followed.

To analyse the nutrient concentrations in *Sphagnum* a sample was taken at the start, at the end and twice during the experiment from every study plot (in October 2006, March 2007, October 2007 and March 2008). At the other two sites nutrient samples were taken every time that the site was visited. After collection the samples were kept cool until, to avoid rinsing away some of the nutrients with water, the samples were thoroughly cleaned of litter using tweezers. Capitula (0-1 cm), and at two sampling times also stem parts (3-8 cm below capitulum), were cut from the stems. The samples were then oven-dried for at least 72 hours in 70 °C and ground with a ball-mill. C and N were measured with Carlo Erba NA2500 total C/N analyser. The October 2006 samples were analysed in a different laboratory using the same method except for being heated once more at 105°C for 1 hour after being ground. In October 2006 and March 2007 also phosphorous (P), potassium (K), magnesium (Mg) and Calcium (Ca) were analysed from the capitula with a Bran+Lubbe Auto Analyser (P) and Solaar M series Unicam Atomic Absorption Instrument (K, Mg and Ca).

To relate the productivity and shoot extension to the visual damage that could be seen in *Sphagnum* all the microplots at Whim were given a score in May 2008. The scoring was subjective but all the assessments were done in one day by the same person to minimise the error. A score between one and five was assigned to every *Sphagnum* microplot. The plot got a score of five when *Sphagnum* looked very healthy and moist and a score of one when *Sphagnum* looked very unhealthy, there was lot of algae, it was very dry, the hummock was falling apart or some other visible damage could be seen (Fig. 4.1).



Figure 4.1. Examples of *Sphagnum* stands from different score classes used in the scoring of the microplots. Score 1 was given to the *Sphagnum* stands that looked very unhealthy (top left) and score 5 to healthy looking *Sphagnum* (bottom right). Top right = score 2, centre = score 3 and bottom left = score 4.

Statistical analysis

Because the visual scores represented a subjective constrained classification system, a non-parametric Kruskal-Wallis test was carried out to establish the significance of differences between the treatments. For the other results the PK addition was treated as a separate study: the effects of N addition, N form, N dose and interactions were tested separately with and without PK. Analyses of variance (ANOVA) was carried out to test the effect of N form and N dose and PK addition for shoot extension, productivity, stem weight cm^{-1} , capitulum density and %N and C:N ratios of both the capitulum and stem parts and for %P, %K, %Ca and %Mg for the capitulum. For the shoot extension data the ANOVA was carried out for the data representing one year only. The second measurement set of %P was excluded from the analysis due to uncertainty in the validity of the results (measured with different

instrument giving very different results). All the variables were tested for normality and homogeneity of variance and log transformed if necessary. For the nutrient sample data the measurement date was used as a covariate in the analysis. All the analyses were carried out using Genstat version 10.

Results

Environmental variables

Both years at the main study site were wetter than average, with 1235 mm rainfall in 2007 and 1400 mm rainfall in 2008 (Fig. 4.2). The minimum temperature during year 2007 was -9.8 °C recorded in December and the maximum temperature 23.5 °C recorded in August; in 2008 the minimum temperature was -7.4 °C recorded in February and the maximum temperature 25.4 °C recorded in July. The minimum water table during the experiment was in the control plots -26.3 cm, in the 'N only' treatments -25.4 cm and in the NPK treatments -40.8 cm. The water table was always below the ground level and the one average measurement above 0 cm in the control plots in January 2008 (Fig. 4.2) is probably due to high rainfall that month (205.6 mm). The water table fluctuation was similar in all plots.

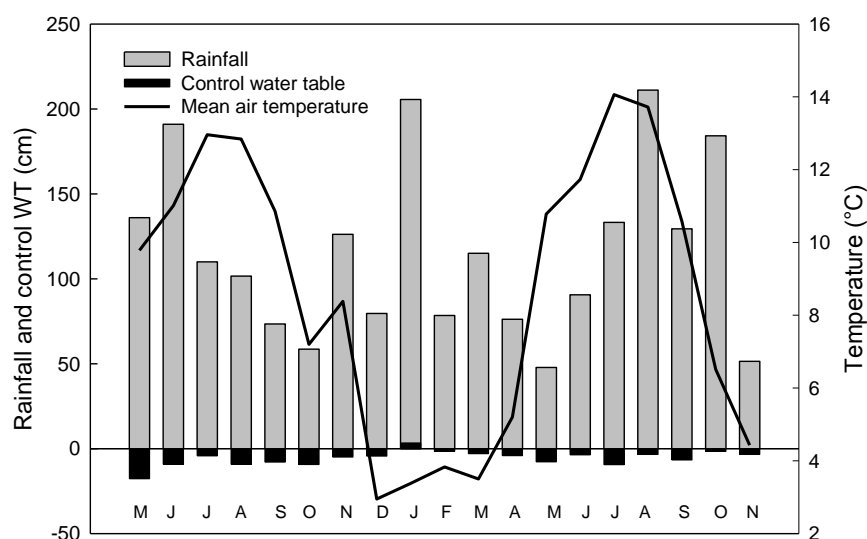


Figure 4.2. The mean monthly air temperature, the total monthly rainfall and water table fluctuation in the control plots (average) between May 2007 and November 2008 at Whim bog.

Visual scoring of the health of *Sphagnum*

Compared to the score for control plots a small increase in nitrate increased the health score both with and without PK but larger nitrate doses lowered the score (Fig 4.3). The Kruskal-Wallis test showed that there were differences between the treatments (Chi-squared probability < 0.001). In the ammonium plots the scores indicated more damage than with nitrate, and as with nitrate scores were lower with increasing N dose. Adding PK with ammonium alleviated the effect and the condition of the *Sphagnum* scored the same as the control or better, *i.e.* PK mitigated the effects. PK additions also improved the score in the nitrate treatments although NO56PK did not reach the control level. The mean rankings of different treatments can be seen in Table 4.2. Also very little *Sphagnum* was growing in the high N, especially in the NO56PK, plots suggesting that *Sphagnum* was disappearing from the high N plots.

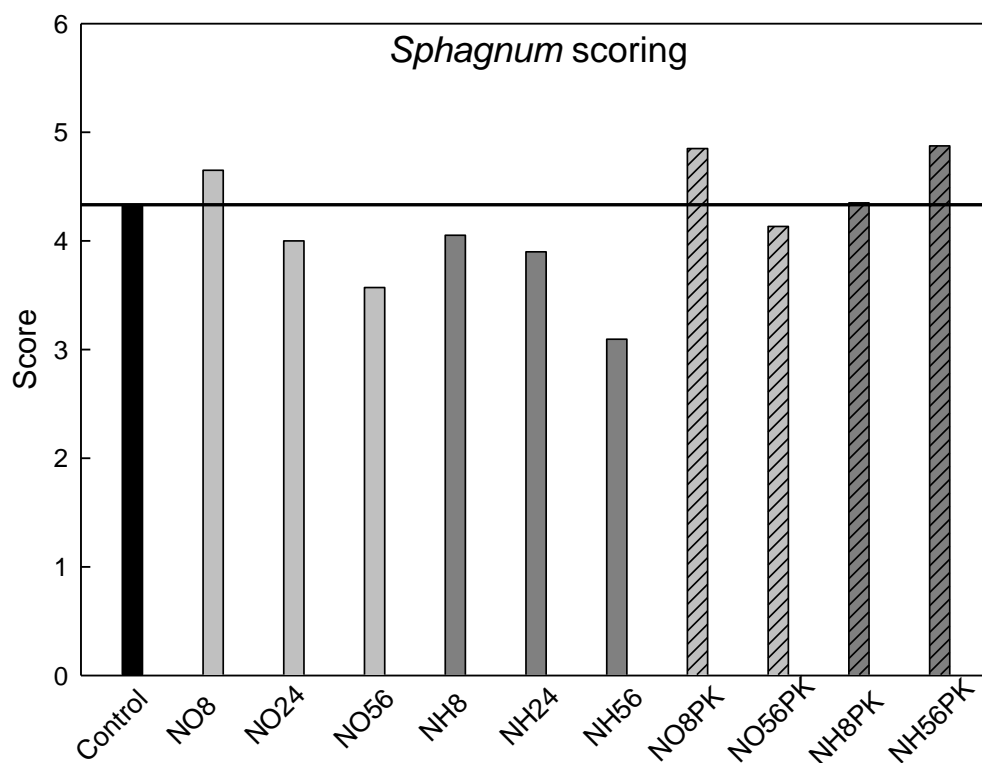


Figure 4.3. The average score for *Sphagnum* healthiness based on visual damage at Whim. Score five was given to stands that looked healthy and score one was given to unhealthy stands (very dry, structure breaking, algae growing or other visible damage seen). The line represents the control level.

Table 4.2. Results of the Kruskal-Wallis test for the healthiness scores in different treatments (Chi-squared probability <0.001).

Treatment	Sample size	Mean rank
NH56PK	24	154
NO8PK	20	151
NO8	20	142
Control	21	119
NH8PK	20	119
NO56PK	15	108
NH8	20	101
NO24	21	100
NH24	21	90
NO56	20	74
NH56	19	59

Shoot extension and productivity

After one year the shoot extension in the control plots averaged 22 mm, in the `N only` treatments 13-17 mm and in the NPK plots 16-27 mm (Fig. 4.4). After one year there was a statistically significant reduction in shoot extension in the `N only` addition treatments; the N dose or the form had no effect (Table 4.3). Adding PK changed the situation and increased the shoot extension compared to `N only` treatments (Table 4.4) . Form alone did not have an effect because in the 8PK plots nitrate increased the shoot extension compared to control but did not in the 56PK plots and an opposite trend was found for ammonium. However, there was a form*dose interaction due to the different response in the nitrate and ammonium treatments. A dose effect when N was added with PK was found.

Table 4.3. Analysis of variance of effects of N addition, N dose, N form and dose*form on shoot extension of *S. capillifolium* at Whim between May 2007 and May 2008.

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
N addition	1	2.2052	2.2052	18.38	<0.001
N dose	3	0.4987	0.1662	1.39	0.276
N form	2	0.4488	0.2244	1.87	0.18
Dose*form	1	0.2626	0.2626	2.19	0.155
Residual	20	2.3989	0.1199		
Total	27	5.8142			

Table 4.4. Analysis of variance of effects of PK addition, N dose with PK, N form with PK, PK*dose, PK*form, dose*form and PK*dose*form on shoot extension of *S. capillifolium* at Whim between May 2007 and May 2008.

Source of variation	d.f	s.s	m.s	v.r	F pr.
PK	1	5.4777	5.4777	25.77	<0.001
N dose with PK	1	0.9723	0.9723	4.57	0.043
N form with PK	1	0.016	0.016	0.08	0.786
PK*dose	1	0.004	0.004	0.02	0.893
PK*form	1	0.7574	0.7574	3.56	0.071
Dose*form	1	2.8453	2.8453	13.39	0.001
PK*dose*form	1	1.1537	1.1537	5.43	0.029
Residual	24	5.1015	0.2126		
Total	31	16.3279			

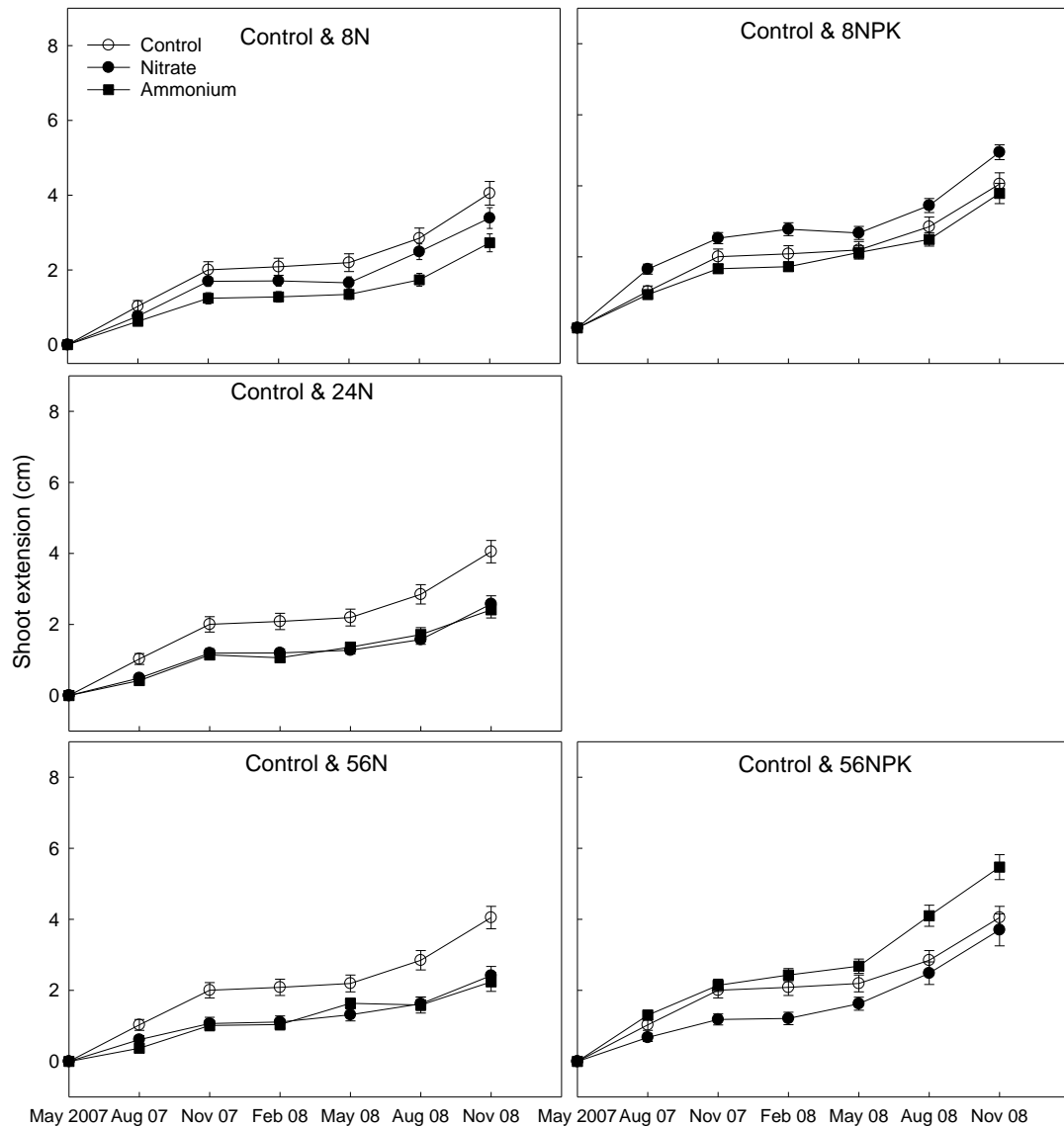


Figure 4.4. Shoot extension \pm SE of *S. capillifolium* at Whim study site between May 2007 and November 2008 in all the treatments.

Adding N decreased productivity compared to the control treatment ($P=0.039$) (Table 4.5, Table 4.6). As in the shoot extension the productivity decreased with increasing N dose, but again this was not statistically significant ($P=0.215$) and there was no form effect. Adding PK with N significantly increased the productivity compared to 'N only' ($P=0.097$) as did the dose of N when added with PK ($P=0.003$) (Table 4.7). No form response or interactions were found.

Table 4.5. The productivity of *S. capillifolium* in the different treatments calculated from the shoot extension, density and stem weight data organised in the order from highest to lowest.

Treatment	Average productivity (g m⁻² y⁻¹)	Standard error
NO8PK	394.6	99.9
Control	243.1	65.7
NH56PK	197.7	71.5
NO8	163.5	34.2
NH8	155.6	50.6
NH8PK	143.5	25.9
NO24	124.5	42.4
NH24	99.8	22.2
NO56PK	85.8	26.8
NH56	82.0	30.5
NO56	72.5	17.5

Table 4.6. Results from probability of effects of N addition, N dose, N form and dose*form on productivity of *S. capillifolium* at Whim between May 2007 and May 2008.

Source of variation	d.f	s.s	m.s.	v.r.	F pr.
N addition	1	0.46672	0.46672	4.89	0.039
N dose	3	0.46583	0.15528	1.63	0.215
N form	2	0.01881	0.0094	0.1	0.907
Dose*form	1	0.0096	0.0096	0.1	0.754
Residual	20	1.90741	0.0953		
Total	27	2.86837			

Table 4.7. Results from probability of effects of PK addition, N dose with PK, N form with PK, PK*dose, PK*form, dose*form and PK*dose*form on productivity of *S. capillifolium* at Whim between May 2007 and May 2008.

Source of variation	d.f	s.s	m.s	v.r	F pr.
PK	1	0.3068	0.3068	3.02	0.097
N dose with PK	1	1.1414	1.1414	11.24	0.003
N form with PK	1	0.1304	0.1304	1.28	0.27
PK*dose	1	0.1146	0.1146	1.13	0.3
PK*form	1	0.1823	0.1823	1.8	0.195
Dose*form	1	0.0096	0.0096	0.09	0.762
Residual	21	2.1324	0.1015		
Total	27	4.0175			

No statistically significant effects of N addition, N dose, N form or PK additions for the capitulum density were found. Adding `N only` did not affect the stem weight but adding NPK decreased the stem weight ($P=0.002$) and the N dose when added with PK had also an effect ($P=0.018$), i.e. the stem weight was even more reduced in the 56PK plots. The effects of N and NPK on shoot extension, stem weight and productivity are summarised in table 4.8.

Table 4.8. All the effects (significant in the $P < 0.05$ level) of N or NPK additions on shoot extension, stem weight and productivity of *S. capillifolium* at Whim study site are designated with an arrow showing the direction of response. No arrow means no significant response.

Source of variation	Shoot extension (cm y ⁻¹)	Stem weight (g cm ⁻¹)	Productivity (g m ⁻² y ⁻¹)
N addition	↓	-	↓
N dose	-	-	-
N form	-	-	-
PK with N	↑	↓	↑ (NH ₄) ↑ (NO ₃)
N dose with PK	↓ (NO ₃) ↑ (NH ₄)	↓	↓ (NO ₃) ↑ (NH ₄)
N form with PK	-	-	-

Plant tissue nutrients

Large differences between the four measurement dates were found even in the control treatments. Such differences can reflect different conditions at the site (amount of rain and treatment and time of year relative to growth) but also for the October 2006 samples a different methodology/analyser was used. The first two measurement sets had higher %N values due to ~45 minutes of treatment applied in the previous 7 days whereas the last two measurement sets had lower values due to not having any treatment applied in the 7 days prior to the sampling. The covariate, measurement date, was significant both with and without PK ($P < 0.001$). However, the treatment trend was exactly the same in all the sets and the data were combined for analysis. The N dose significantly ($P < 0.001$) increased %N in the capitulum, and with ammonium %N was significantly higher ($P < 0.001$) than with nitrate (Fig. 4.5). There was also a dose*form interaction ($P = 0.023$) due to ammonium increasing %N more for the same dose. The C:N ratio reflected %N. Adding PK significantly

decreased ($P < 0.001$) %N compared to 'N only' treatments leading to an increased C:N ratio. There was also a form ($P = 0.07$) and dose ($P < 0.001$) response found when N was added with PK.

Also in the stem parts the %N increased with the N dose ($P < 0.001$) but it did not reach such high levels as in the capitula (Fig. 4.5). The ratio between %N in the capitulum and %N in the stem did not change when the N dose was increased implying that the stem was used for storage but that the relative proportion stored in the stem remained the same over time. In the stem parts no form effect was found although ammonium increased %N more than nitrate with high N doses; there was also a dose*form interaction found ($P = 0.047$). Similarly to the capitula, the %C was very similar in all the stem samples and the C:N ratio followed changes in the %N. There was no change in the stem C:N ratio compared to capitulum C:N ratio, again indicating that the proportion of N that was stored in the stem was similar in all treatments. Adding PK decreased the stem %N ($P < 0.001$) compared to 'N only' treatments. Increasing N dose when added with PK increased stem %N ($P < 0.001$). There was also a form*dose interaction found for stem %N ($P = 0.037$).

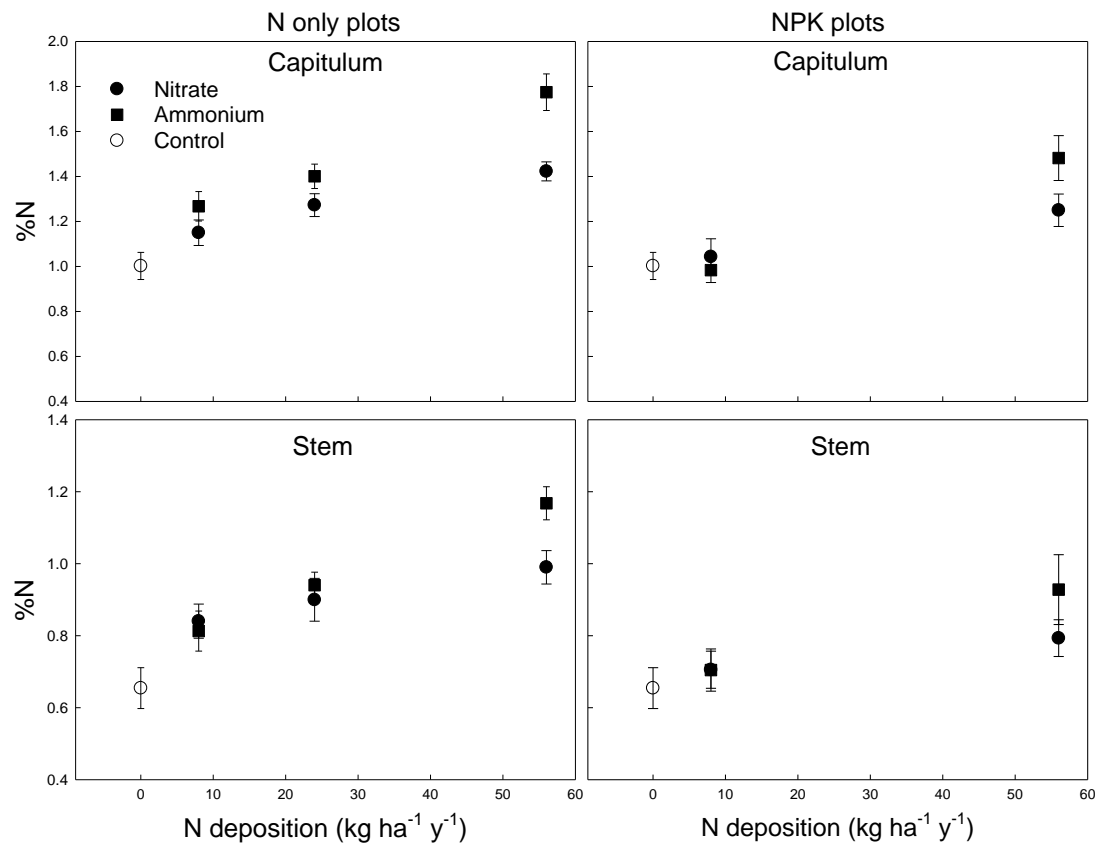


Figure 4.5. %N \pm SE for capitulum (0-1 cm) and stem part (3-8cm) at Whim. The value is an average from all four measurements sets (four plots per treatment in every set) during the experiment.

N addition increased %P ($P=0.022$) in the capitula as did NPK additions ($P<0.001$). N addition also increased %K ($P=0.018$) as did NPK additions ($P<0.001$) and with PK also a dose response ($P=0.004$) and form response ($P<0.001$) were found nitrate increasing %K more than ammonium. When NPK was added a PK*dose interaction ($P=0.002$) was found. All the plots had high N:P and N:K ratios, suggesting P- and/or K-limitation (Fig. 4.6, Table 4.9) and, as expected, adding 'N only' further increased the N:P and N:K ratios. Adding PK with N caused them to fall below the suggested N:P ratio that suggests N-limitation (Koerselman & Meuleman 1996). The N:K ratio was never below the suggested N-limitation limit even in the PK or control plots indicating K-limitation.

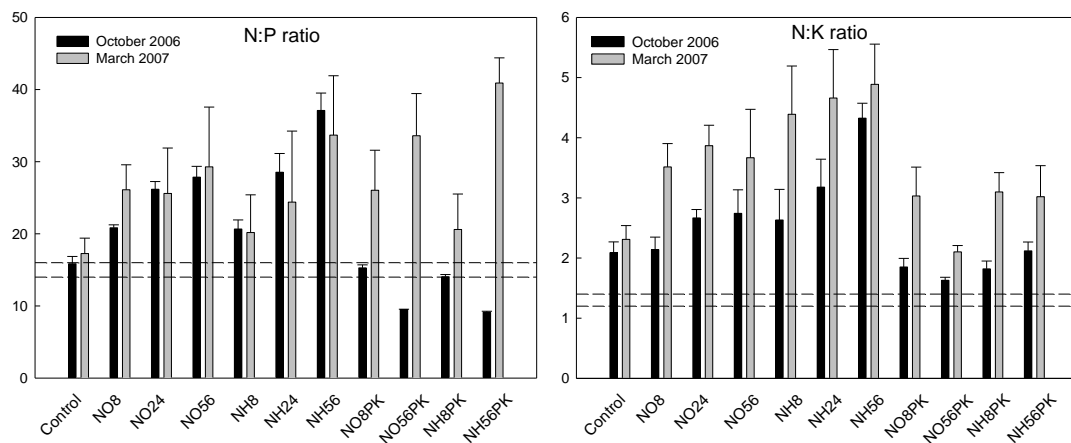


Figure 4.6. Average N:P \pm SE and N:K \pm SE ratios at the different treatments. The lines represent the suggested limits by Koerselman & Meuleman (1996) above which *Sphagnum* would be P- or K-limited and below which it would be N-limited. In between a co-limitation would occur.

Table 4.9. Capitulum (cap.) (0-1 cm) and stem (3-8 cm) C:N ratios \pm SE and their relationship at Whim study site.

Treatment	C:N cap. \pm SE	C:N stem \pm SE	Cap. C:N /stem C:N
C	43 \pm 4	67 \pm 6	65
NO8	36 \pm 3	51 \pm 3	70
NO24	32 \pm 1	49 \pm 3	65
NO56	29 \pm 2	43 \pm 3	67
NH8	33 \pm 3	54 \pm 4	61
NH24	29 \pm 2	46 \pm 2	63
NH56	23 \pm 1	36 \pm 1	64
NO8PK	41 \pm 5	62 \pm 4	66
NO56PK	31 \pm 2	54 \pm 4	58
NH8PK	45 \pm 4	63 \pm 6	72
NH56PK	29 \pm 3	50 \pm 6	58

No statistically significant N addition, dose or form effects were found on %Ca (Fig. 4.7) but adding NPK had a decreasing effect on %Ca ($P=0.018$) compared to 'N only' treatments. N addition or N dose had no effect on the amount of %Mg but N form had a significant effect ($P<0.001$); ammonium decreased the amount of %Mg more than nitrate. Adding PK with N had a decreasing effect on the amount of Mg ($P=0.01$) compared with N alone and also in the presence of PK ammonium decreased %Mg more than nitrate ($P<0.001$).

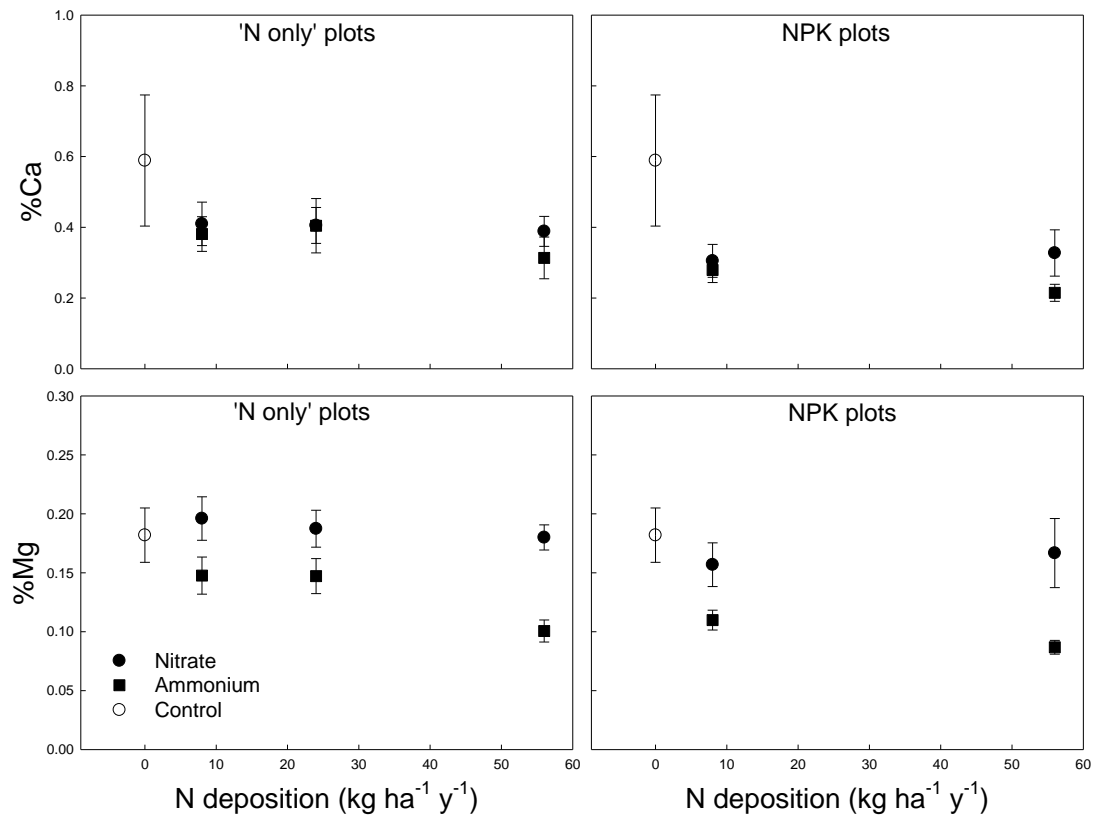


Figure 4.7. %Ca \pm SE and %Mg \pm SE in the capitulum (0-1 cm) of *Sphagnum capillifolium* at Whim. The value is an average from two different measurements during the experiment.

Whim vs. other sites

Shoot extension over 12 months was similar for Whim control plots and Moor House and less in Cairngorms (Table 4.10). This can be expected due to the much colder conditions and longer period with snow at Cairngorms. However, capitulum density was much lower at Whim compared with the other two sites (Fig. 4.8) The coverage of the two subspecies (*rubellum* and *capillifolium*) was not recorded at the two study sites and therefore it is not possible to assess if this difference in the density is due to different coverage of the subspecies at each site. Stem weight was higher at Moor House than at Whim but much lower at Cairngorms. These differences in the components led to much higher productivity at Moor House than at Whim or Cairngorms.

Table 4.10. Average shoot extension \pm SE, density \pm SE, stem weight \pm SE and productivity \pm SE at Whim control plots compared to the other two study sites.

Site	Shoot extension (cm y ⁻¹)	Density (individuals m ⁻²)	Stem weight (g cm ⁻¹)	Prod. (g m ⁻² y ⁻¹)
Whim	2.2 \pm 0.24	16600 \pm 1710	0.0064 \pm 0.0007	243 \pm 65.7
Moor House	2.5 \pm 0.26	22900 \pm 1640	0.0088 \pm 0.0017	503 \pm 76.6
Cairngorms	1.5 \pm 0.24	25800 \pm 2090	0.0049 \pm 0.0004	173 \pm 29.2

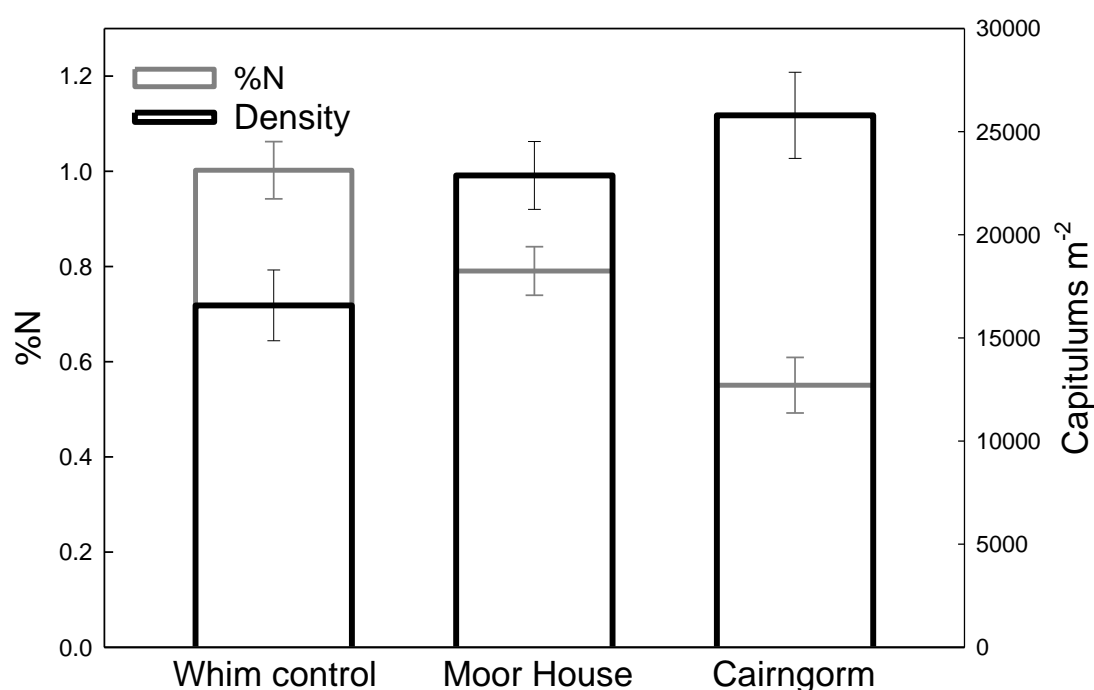


Figure 4.8. Capitulum N concentrations \pm SE and capitulum density at Whim and the other two sites calculated as an average for all the measurements carried out at each site.

The %N in the capitulum was higher in the Whim control treatment than at Moor House or Cairngorms (Fig. 4.8). Accordingly, the C:N ratio was lower at Whim than at Moor House and at Cairngorms it was almost twice as high. The amount of P and K in the capitula was also lower at the additional sites as were the concentrations of base cations. The N:P ratio was very high at the two additional sites indicating P-limitation (Table 4.11).

Table 4.11. Average nutrient concentrations and ratios in the capitulum of *Sphagnum capillifolium* \pm SE from 3 northern UK sites: Whim (W), Moor House (MH) and Cairngorms (C).

Site	%N	%P	%K	%Ca	%Mg	N:P	N:K
W	1.002 \pm 0.06	0.07 \pm 0.01	0.53 \pm 0.04	0.58 \pm 0.2	0.18 \pm 0.02	17 \pm 1	2.2 \pm 0.14
MH	0.791 \pm 0.05	0.03 \pm 0.002	0.28 \pm 0.06	0.36 \pm 0.05	0.17 \pm 0.03	35 \pm 3	3.6 \pm 0.77
C	0.551 \pm 0.06	0.02 \pm 0.007	0.43 \pm 0.1	0.22 \pm 0.01	0.11 \pm 0.01	39 \pm 15	1.5 \pm 0.28

Discussion

How do we know whether enhanced N deposition is having an adverse effect on S. capillifolium?

Visible damage proved to be a good indicator of effects of increased N on *S. capillifolium*. Increasing N deposition decreased the score given for *Sphagnum* vitality with lower scores corresponding to decreased shoot extension and productivity in the 'N only' plots and higher scores for NPK plots reflected the improvement in shoot extension in NPK plots compared to 'N only' plots. Evidence of N induced damage includes increases in algal slime, capitula that are easily dislodged from their stems and the hummocks appear loose *i.e.* less dense. No specific visible symptoms associated with N form were observed although *Sphagnum* receiving ammonium was less vital than those with nitrate. Damage was driven by N dose, only the low, 8NO treatment with and without PK and NH56PK showed no sign of decline.

Looking at the data from the three study sites we can see an inverse relationship between %N and capitulum density. It could be that the amount of different subspecies (*rubellum* and *capillifolium*) at the different study sites is different which could lead to difference in the density as var. *capillifolium* is denser than var. *rubellum*. However, no apparent difference in the coverage of the two subspecies was found (pers.obs.). A similar relationship was found among the N treatments at Whim (although not statistically significant), where increasing %N was correlated with lower density. The fact that the difference was also found at Whim also supports

the interpretation that the difference in density is to do with %N rather than subspecies. This might be because N deposition decreases *Sphagnum* density or, the low density could be the reason for high %N (less mass to take up the deposited N). Hummock density could possibly be used as an indicator of N damage to *Sphagnum* as previously suggested by Carfrae (2004) based on a greenhouse study on N effects on shoot branching. This observation suggests capitulum density is a factor that should be considered when sampling *Sphagnum* for the purpose of relating %N to N deposition; measuring capitulum density could be used to reduce the ‘noise’ in the data.

Changes in *Sphagnum* morphology with increased N were observed in the first few years of N fertilization at this site, measurements revealed that shoot branching of *Sphagnum* was reduced in the high N plots (Carfrae *et al.* 2007). Our observations at Whim are in good agreement with those seen in other N manipulation studies where *Sphagnum* have been found to lose their form, have more fragile stems that ‘break’ easily allowing the capitula to be dislodged, have capitula that change colour to brown or black *i.e.* lose their light harvesting capability, have increasing amount of algae growing over them and hummocks that collapse (Gunnarsson & Rydin 2000; Van der Heijden *et al.* 2000; Limpens *et al.* 2003b). Given the real world treatment scenario used at Whim we conclude that such symptoms are indicative of N impacts in the field. However, for any assessment tool to be useful for evaluating the state of *Sphagnum* regarding N deposition in practice, which can be used over large areas, the assessment should be very easy to carry out and affordable. Assessing visible damage fulfils these criteria at least for *Sphagnum capillifolium* and possibly for other hummock species; lawn and pool species of *Sphagnum* were not assessed in this study and the visible symptoms described here may not necessarily be expressed in such species.

N effects – do N form and N dose matter?

Shoot extension growth, between 13 and 27 mm, was similar to results found in other studies (Rocheffort *et al.* 1990; Limpens *et al.* 2003a). As in many other studies,

adding N in excess decreased shoot extension of hummock forming *Sphagnum* (Gunnarsson & Rydin 2000; Berendse *et al.* 2001; Limpens *et al.* 2003a; Limpens *et al.* 2004) and, in contrast to the hypothesis, this happened at even small doses. A form effect was expected as ammonium is more readily available for *Sphagnum* and taken up more effectively than nitrate (Jauhiainen *et al.* 1998). Accordingly, ammonium increased %N more than nitrate which is also supported by the results by Wiedermann *et al.* (2009) who found that most N is taken up as ammonium. Also Carfrae *et al.* (2007) found in a study at the same site 5 years earlier that ammonium increased capitulum %N more than nitrate. However, the greater uptake of ammonium did not cause greater reductions in shoot extension than nitrate; both N forms reduced extension equally, although in the 8kg plots *Sphagnum* grew more in the nitrate compared to the ammonium plots. Findings that nitrate has a more negative effect on the amount of photosynthesising material than ammonium was also reported in the early years of this experiment (Carfrae *et al.* 2007). One possible explanation for the similar effects of both N forms regardless of higher %N with ammonium is that a lot of ammonium can be bound extracellularly where it is less challenging to the physiology of *Sphagnum*. However, higher concentrations of amino acids were measured in the ammonium treated capitula (van den Berg L., unpublished) indicating the excess N had been ‘detoxified’ but was intracellular. The negative effects of nitrate, associated with less photosynthetic material suggests, that nitrate is interfering at the cellular level with carbon assimilation, possibly with light harvesting.

The maximum level, to which N can accumulate in *Sphagnum*, before being leached into the pore water, has been suggested as 2% dry weight (Berendse *et al.* 2001). However, here as also reported by Limpens *et al.* (2003a), the amount of N in the capitulum increased linearly with N deposition to above the suggested threshold of 2%. In some studies it has been found that when the N deposition is very high *Sphagnum* starts storing excess N in the stem instead of the capitulum to avoid toxic effects (Limpens *et al.* 2003b; Bragazza *et al.* 2005). Also in the present study storage was detected, but the ratio between the amount of N in the capitulum and stem remained the same indicating that a constant proportion of N was stored in the

stem irrespective of N dose. Both this and the high %N imply that *Sphagnum* has no protective mechanism against N accumulation and from Berendse *et al.* (2001) we expect intracellular N accumulation to reach a concentration whereby membrane integrity is compromised and N and other constituents start to leak out. Pore water measurements made during this study showed that the amount of N and especially organic N did increase in the pore water collected from within hummocks in the high N plots, especially in the NO56 plots (Paper II). These observations appear to confirm that at least in the high N dose plots the integrity of the *Sphagnum* has been compromised, concurring with the visible damage.

Productivity values between 72-395 g m⁻² y⁻¹ measured in this study were similar to those measured in other studies (Rocheffort *et al.* 1990; Gunnarsson & Rydin 2000). Decreased productivity due to excess N has been found in many studies (Gunnarsson & Rydin 2000; Berendse *et al.* 2001; Limpens *et al.* 2003a; Limpens *et al.* 2004). The form of N as found for shoot extension, made no significant difference to the N effect on productivity, but increasing the N dose decreased productivity. Productivity is made up of several components which may be differentially sensitive to N. Neither capitulum density nor stem weight were significantly affected by N although a decrease in capitulum density was found with increasing N. In other studies either lower capitula density in high N (Bragazza *et al.* 2004) or no change was found (Jauhiainen *et al.* 1994; Gunnarsson & Rydin 2000). As no other changes were found, in the 'N only' plots the reduced productivity reflected the decrease in shoot extension.

Does improving PK availability alleviate N effects?

According to the hypothesis the reduction of shoot extension caused by increased N was alleviated when PK was added with N indicating that P and K availability can compromise the response to increasing N deposition, as found also by Aerts *et al.* (1992) and Limpens *et al.* (2004). Bragazza *et al.* (2004) found that N:P and N:K ratios reached saturation at a deposition level of around 10 kg ha⁻¹ y⁻¹ but in this study the N:P and N:K ratios increased linearly and did not level out. Adding N

increased %P and %K but no significant form or dose response was found. Possible explanations for the increase in P and K are increased mineralization and enzyme production *e.g.* phosphatase (Phuyal *et al.* 2008) with increased N, due to faster turnover of plant material and nutrient release, or less growth dilution due to decreased growth, as observed. Adding PK with N continues to have an alleviating effect on the %N, previously reported by Carfrae *et al.* (2007). In the present study %N remains at the same level in the NPK plots as in the control plots. This is likely to be due to increased growth in the NPK plots compared to 'N only' plots that leads to dilution of N.

Productivity was affected by NPK additions due to decreases in stem weight. In the NPK plots the decreased stem weight was compensated by increased shoot extension in the NO8 and NH56 plots but not in NO56 and NH8 where productivity decreased. The effect of PK when added with N on productivity is two-fold. When added with a small amount of nitrate or a large amount of ammonium, it improved the productivity but in the other two NPK treatments it had a reverse effect. An indication of PK improving the situation was also found in CO₂ fluxes measured from the same plots where adding NPK increased gross photosynthesis compared to 'N only' (Paper III). The decrease in the shoot extension and productivity in the NO56PK plots was probably due to very large expansion of *Hypnum jutlandicum* in this treatment that led to overgrowth and suffocation of *Sphagnum*. It seems that nitrate together with PK would be advantageous for *Sphagnum* growth and productivity but when added in large amounts together with PK it is even more advantageous for *Hypnum*, which outcompetes *Sphagnum*. *Has S. capillifolium acclimated to elevated N?*

The lack of N dose effect on shoot extension suggests that at Whim bog the 5 years of N additions, even as low as 8 kg N ha⁻¹ y⁻¹, have already exceeded the growth requirement for *Sphagnum* leading to decrease in shoot extension. The background deposition at Whim is 8 kg ha⁻¹ y⁻¹ which is within the critical load (CL). The present study supports this relatively low CL of 5-10 kg N ha⁻¹ y⁻¹ for bogs, given the importance of *Sphagnum* to their sustainability and ability to provide ecosystem services, such as C sequestration. Based on their study Limpens *et al.* (2003b)

suggested that *Sphagnum* can adapt to enhanced N deposition. However, this present study contradicts this. After six years of fertilizing, the growth and productivity of *Sphagnum* are decreased and in fact, *Sphagnum* is actually disappearing from the high N plots. If hummock forming *Sphagnum* cannot adapt to enhanced N deposition above the CL, their conservation will rely on the strict enforcement of controls on emissions.

How representative are these results?

Both years 2007 and 2008 were very wet, especially 2008, when the rainfall was ~50% above average at the Whim bog study site providing ideal conditions for *Sphagnum* growth. However, the high rainfall and consequent high N deposition during the study years might affect the results and cause more damage than would be seen on an average year. Carfrae *et al.* (2007) found that all the negative effects of increased N on *Sphagnum* were exaggerated during drought which suggests that in dry years the negative effects of enhanced N deposition found in the present study, *e.g.* reductions in shoot extension and productivity, could be even larger.

When interpreting these results it has to be taken into account that ombrotrophic *Sphagnum* species have been found to be more vulnerable to N than minerotrophic species and that hummock species take up more N than lawn and hollow species (Jauhiainen *et al.* 1998). Therefore the studied species *S. capillifolium*, might suffer more than *Sphagnum* growing in lawns or hollows at the same study site or minerotrophic *Sphagnum* species. Whim is also an N 'clean' site and therefore the plants are not acclimated to high N and might suffer more than *Sphagnum* in areas with longer exposure time.

Besides inducing toxic effects, increasing N deposition can decrease *Sphagnum* growth indirectly through eutrophication favouring increase in vascular plant cover. We have already provided evidence that N impacted *S. capillifolium* on this site is no longer 'mopping up' all the added N. Berendse *et al.* (2001) found that changes in *Sphagnum* productivity were caused by increased cover of living and dead parts of

other plants that reduced light for *Sphagnum*. At Whim bog, although the cover of *Calluna vulgaris* has increased in the N treatment plots, especially in the NH56 plots (Appendix V), the microplots used in this study were mostly from open hummocks which were not encroached upon by vascular plants. *Calluna* provides shelter and structural support for *Sphagnum* and therefore increase in its cover due to increased N deposition could counteract some of the negative effects of N deposition on *Sphagnum*. This is especially important because as found in the present study increased N deposition breaks up the structure of *Sphagnum* and structural support would therefore be beneficial. However, as mentioned earlier in this study the microplots were chosen from as open areas as possible with very little *Calluna* cover and there was no indication of *Sphagnum* in the NH56 plots doing better compared to NO56 but they were actually worse affected.

Whim vs. other sites

The other two sites at Moor House and Cairngorms were above the P-limitation threshold suggested by Koerselman & Meuleman (1996) (very high N:P ratio). The smaller shoot extension at these two sites compared to Whim might have been caused by lower amounts of nutrients available and lower temperatures; Whim also had more available cations. However, the productivity at Whim was lower regardless of the greater shoot extension than at Moor House. This was caused by lower density of *Sphagnum* at Whim which might reflect Whim being drier and the hummocks being higher and more likely to dry out. The much higher %N at Whim than at Moor House and Cairngorms is unexpected because the background N deposition is lower at Whim than at Moor House and not much higher than at Cairngorms. The explanation for this might be the substantially (27 % and 35%) lower capitulum density which means less mass of *Sphagnum* per unit area to take up the deposited N and so that per mass, more N is being taken up. The proportion of oxidised and reduced N is different at the three sites but does not seem to have big impact which agrees with the results from Whim where no difference between ammonium and nitrate was found in shoot extension and productivity. The much lower %N at Cairngorms might be due to lower N deposition but also the lower proportion of

reduced N. The higher amounts of other nutrients at Whim might be due to the close proximity of the sea and dust from the tracks around the Whim study site.

We conclude that reducing N deposition at least down to the critical load is crucial to the well being of Scottish bogs. Because no form effect was found on shoot extension and productivity but the N dose was crucial, emissions from sources of both oxidised and reduced N should be targeted.

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5. Paper II “Increased nutrient deposition slows down decomposition of *Sphagnum* and increases the amount of N in the pore water in an ombrotrophic bog”

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Summary

The increase in N deposition caused by anthropogenic activities might change the decomposition rate of the main peat formers in northern bogs, the *Sphagnum* mosses, and therefore affect their C sink strength. The aim of the experiment was to look at the effects of increased nutrients (N with/without P and K) on the decomposition of *Sphagnum capillifolium* using a combination of litter bags (mass and nutrient loss) and rhizon samplers to determine the amount of DOC and N in the pore water. The study was carried out at Whim bog in southern Scotland where a long term (2002 onwards) nutrient addition experiment is running. The experiment had study plots where different rates (8, 24 and 56 kg ha⁻¹ y⁻¹) and forms (ammonium and nitrate) of N have been added, and control plots.

In all the treatments adding N and NPK decreased mass loss compared to control, this was statistically significant after 6 and 12 months (P=0.026 after 6 months, P=0.002 after 12 months and P=0.062 after 18 months). No effect of N form or dose was found. In the beginning no difference between NPK and ‘N only’ treatments was found but after 18 months adding PK with N increased, although not significantly, the decomposition compared to ‘N only’ treatments (P=0.07). No significant effect of N addition, N form or N dose on DOC in the pore water was found but in the PK treatments there was a response to the form of N (P=0.022), i.e. when nutrient limitation was removed for the high N dose, treatment with nitrate increased DOC whereas ammonium reduced DOC. Adding N, especially nitrate increased the amount of N in pore water.

Introduction

It has been estimated that about 455Pg of carbon is stored in boreal peatlands, which amounts to one third of the global soil carbon pool (Gorham 1991; Post *et al.* 1982) and annually northern peatlands accumulate 0.07 Gt of C (Clymo *et al.* 1998). It is specifically the slow decomposition rate, rather than high productivity, that drives C accumulation and peatland formation (Clymo 1983). The slow decomposition rate in these ecosystems is due to low soil temperatures, low pH, low nutrient inputs and subsequent low quality of plant litter, and the anoxic conditions of the lower peat layer, the catotelm, caused by the high water table (Van Breemen 1995; Aerts & De Caluwe 1997; Robinson 2002; Lindsay 2010).

In ombrotrophic bogs *Sphagnum* mosses are the main peat forming vegetation. *Sphagnum* mosses have morphological and physiological properties (for example their organochemical composition and high cation exchange capacity) that create nutrient poor, acidic conditions that favour their sustainability and restrict their decomposition causing them to be described as ‘ecological engineers’. By restricting decomposition through the maintenance of conditions unfavourable to macro and micro-organisms *Sphagnum* also ‘locks up’ other nutrients in addition to C, such as N and P that are normally mineralised (Van Breemen 1995). Thus on most *Sphagnum* dominated peatlands vascular plant cover is restricted with *Sphagnum* species forming the bulk of living and dead biomass (Clymo & Hayward 1982; Van Breemen 1995). Because of the nutrient poor nature of these ecosystems, bog species and especially *Sphagnum* mosses have low physiological tolerance of higher N conditions (Chapin *et al.* 2004), although there are reports of acclimation to enhanced N inputs (Press & Lee 1986). In areas where N deposition is low, $< 10 \text{ kg N ha}^{-1} \text{ y}^{-1}$, *Sphagnum* mosses are able to take up most atmospherically deposited N (Berendse *et al.* 2001; Lamers *et al.* 2000). However, in the last century the anthropogenic N deposition has increased in the northern hemisphere three to five fold (Reay *et al.* 2008). In the UK, the total annual deposition of N averages to $\sim 17 \text{ kg ha}^{-1} \text{ y}^{-1}$ and in certain areas in Europe the deposition can reach $60 \text{ kg ha}^{-1} \text{ y}^{-1}$. These rates of deposition significantly exceed the critical load of N for ombrotrophic

bogs, estimated to be 5-10 kg ha⁻¹ y⁻¹ (Bragazza *et al.* 2004; Bobbink & Roelofs 1995). Because potential decay rates of *Sphagnum* litter are strongly controlled by N and P availability (Aerts *et al.* 2001) the higher N concentration in the *Sphagnum* tissue produced by increased N deposition compared to C and P is expected to change the decomposition rate of *Sphagnum* and peat accumulation (Aerts *et al.* 1992).

Some studies have found faster decomposition of *Sphagnum* with higher N deposition (Aerts *et al.* 2001; Limpens & Berendse 2003; Bragazza *et al.* 2006). This has been linked to increased amounts of easily degradable and mobile amino acids in *Sphagnum* growing under elevated rates of N deposition (Limpens & Berendse 2003a). It has also been suggested by Bragazza & Freeman (2007) that increased N deposition leads to decreased amounts of polyphenols that normally inhibit the decay of *Sphagnum*. On the other hand, it is possible that increasing N in the litter can lead to a shortage of P or K and thus limit the decay (Aerts & De Caluwe 1997; Hogg *et al.* 1994; Hoosbeek *et al.* 2002). Therefore, it has been hypothesised that when N deposition increases, P availability takes over as the controlling factor influencing litter decay rates (Bragazza *et al.* 2006; Aerts *et al.* 2001). Hoosbeek *et al.* (2002) suggested that no changes could be seen in the rate of decomposition with increased N deposition because many bogs already are K-limited or K- and P-co-limited and with the increasing N deposition all bogs would become P or K limited. Accordingly, they found no difference in decomposition rates with increased N contents with *S. magellanicum*, *S. balticum* and *S. fallax*. These authors stressed the importance of P, over N with respect to decomposition, based on rates of CO₂ evolution in laboratory studies. Further uncertainty is associated with the timescale over which the effects of changes in nutrient concentration may be relevant for decomposition and whether or not responses are species specific. There are differences in the decomposition rate between *Sphagnum* species and the microhabitats they occupy within peatlands; in general species occupying hollows decay faster than those on hummocks (Belyea 1996; Limpens & Berendse 2003b). However, Limpens & Berendse (2003b) suggested that the species differences disappear with time, *i.e.* once the litter is more decomposed. In their experiment young stem parts from the hummock species *S.*

magellanicum and *S. papillosum* decayed more slowly than those of the hollow species *S. cuspidatum* and *S. fallax* but the difference was absent in old stem parts.

Increased decomposition of plant material can be expected to lead to increasing concentrations of DOC in the pore water. Increasing N deposition would also be expected to lead to an increase in the amount of N in the pore water. A three phased mechanism has been suggested for the effects of N deposition on *Sphagnum* dominated peatlands (Lamers *et al.* 2000; Berendse *et al.* 2001; Limpens *et al.* 2003). During the first phase *Sphagnum* mosses are able to take up all the deposited nutrients with little transfer of N to the pore water and the rhizosphere. In the second and third phase *Sphagnum* mosses become saturated and are unable to sequester all the deposited N which starts to leak into the rhizosphere becoming available for vascular plants and soil microbes. Support for this has been found by Limpens *et al.* (2003) who found an increase in inorganic N, predominantly ammonium, in pore water of a peat with increasing N (0, 40 and 80 kg ha⁻¹ y⁻¹ in total).

Whether the decomposition increases or decreases with increased N deposition is a question of considerable ecological importance. Accelerated decomposition of *Sphagnum* tissue under elevated N deposition, with decreased growth rates, can result in reductions in the C sink strength of peatlands or in the most extreme case a switch from being a net C sink to becoming a C source. We hypothesized that 1) increased N accelerates the decomposition of dead *Sphagnum* material 2) there would be a greater change in the C:N ratio of decomposing litter in the high N treatments as a consequence of faster consumption of N than C, 3) there would be higher concentrations of DOC in the pore water of high N treatments due to faster decomposition, 4) there would be higher concentrations of N in the pore water in the high N treatments due to less efficient removal by the *Sphagnum*, and 5) increasing the P content, reducing the N:P ratio, would increase mass loss from litter bags.

Material and methods

Study sites

The main study site was Whim Moss. It is an ombrotrophic bog situated c. 30 km to the south of Edinburgh, Scotland (3°16'W, 55°46'N) and represents a transition form between a lowland raised bog and a blanket bog. It is situated 280 m above sea level and has an annual rainfall of c. 900 mm, which is on the dry side for British bogs (Lindsay 1995; Ratcliffe 1964). However, the years 2007 and 2008 when this experiment was performed were very wet with a rainfall of 1235 mm in 2007 and 1400 mm in 2008. The background N deposition is relatively small, approximately 8 kg ha⁻¹ y⁻¹. The vegetation is dominated by *Calluna vulgaris*, *Erica tetralix*, *Hypnum jutlandicum* and *Sphagnum* mosses Sheppard *et al.* (2004).

Whim bog has been used for a long term study (2002 to the present) of the impact of N deposition by manipulating the N dose in wet deposition to examine the effects of different N loads and N forms (reduced and oxidized) (Sheppard *et al.* 2004). In the experiment, N was added to 40 plots either as nitrate (NaNO₃) or ammonium (NH₄Cl) at rates of 8, 24 and 56 kg ha⁻¹ y⁻¹ over and above the background deposition (from now on referred to as 'N only' plots). Some plots, with N loads of 8 kg ha⁻¹ y⁻¹ and 56 kg ha⁻¹ y⁻¹ also had P and K added with N (from now on referred to as NPK plots). P and K were added as K₂HPO₄ maintaining a P:N ratio of 1:14 (8 kg ha⁻¹ y⁻¹ plots received 0.6 kg ha⁻¹ y⁻¹ of P and 56 kg ha⁻¹ y⁻¹ plots received 4 kg ha⁻¹ y⁻¹ of P). There were also four control plots that received additional water only. The additional N was added to rain water collected on site into two 1000 litre storage tanks and enriched with N from bottles of concentrate. The individual plots were supplied by 100 m lengths of plastic pipe joined to a central rotating disc at the centre of every plot. The treatments increased precipitation on the plots by ~10% and spraying was controlled by the volume of rain and wind speed. N additions occurred when it was raining and the concentrations of nitrate and ammonium in the spray did not exceed 4 mM. Over 120 applications took place each year distributed over the whole year provided the air temperature was above freezing. A more detailed

description of the study site can be found in Sheppard *et al.* 2004. At the site dip wells were inserted into the study plots to get an estimate for water table. However, the water table did not represent the moisture level in the hummocks accurately and to get a more reliable estimate for the wetness of *Sphagnum* a fresh weight/dry weight ratio was measured between September 2007 and August 2008 twice a month simultaneously with water tables.

In order to relate the results from the Whim experimental site to other northern bogs two additional sites were included. These additional sites provided differences in natural N deposition as well as in environmental factors, such as rainfall, altitude and temperature (Table 5.1). The study sites belong to the environmental change network (ECN) and have detailed background information available, concerning meteorological and chemical drivers of change. These sites were located at Moor House in northern England and in the Cairngorms in northern Scotland. The Moor House study site is situated in the North Pennine uplands of England (2°45' W, 54°65'N). The total area of the site is 1 500 ha and there is extensive but low intensity sheep grazing in the area (Cundill *et al.* 2007). The study area was situated in a part of the Tees river catchment covered by blanket mire at the altitude of 555 m (Cundill *et al.* 2007). The area was heavily eroded in the 1950s but has recovered since (Cundill *et al.* 2007). The peat depth was on average 2-3 m and the vegetation was dominated by *Calluna vulgaris*, *Eriophorum vaginatum* and *Sphagnum* mosses (Rennie & Lane 1997). The mean annual temperature was 5.3 °C and the mean annual rainfall 1982 mm (Rennie & Lane 1997). Between 1994 and 2003 the average annual N deposition was $10.3 \pm 1.3 \text{ kg ha}^{-1} \text{ y}^{-1}$ with just over half deposited in the reduced form (NH₃) (Cundill *et al.* 2007; Rennie & Lane 1997). The Cairngorms study site was situated high in the Cairngorms, near Aviemore in Speyside (3° 49'W, 57° 06'N) in the Allt a' Mharcaidh catchment. The site covers a total area of 1000 ha and the vegetation comprised mainly *Calluna vulgaris*, *Eriophorum vaginatum*, *Cladonia portentosa* and *S. capillifolium*. Mean annual temperature and rainfall were respectively 5.5°C and 585 mm (1999 to 2009). N deposition was $6.2 \text{ kg ha}^{-1} \text{ y}^{-1}$, with most as oxidized N (Rennie & Lane 1997). At these two sites three plots were chosen from similar vegetation to Whim, ensuring an abundance of *S. capillifolium*.

Table 5.1. Environmental variables of the study sites.

Variable	Cairngorms	Whim	Moor House
Total N deposition (kg ha ⁻¹ y ⁻¹)	6	8	10
Oxidised:reduced N ratio	75:25	50:50	40:60
Rainfall (mm)	585	900	2055
Altitude (m)	750	280	555
Mean annual temperature (°C)	5.2	7	6

Field and laboratory measurements

At Whim the *Sphagnum* material for litter bags was collected in the beginning of March 2007 and at the other sites in June/July 2007. The samples were thoroughly cleaned with tweezers and the stem part (3-8 cm below top of the capitulum) was cut off. The part of the stem used for the experiment was chosen on the basis of the colour and texture of the stem. At this depth (3 cm below the capitulum) the moss changed from green to fawn indicating that it was not photosynthetically vital tissue and could be assumed to be decomposing. The stem parts from each plot were then divided into five portions. One portion was oven-dried for 48 hours at 70 °C, ground with a ball mill and analysed for C and N with a Carlo Erba NA2500 total C/N analyser. The remaining four portions were air-dried for at least 76 hours before being transferred to litterbags. The litterbags were made out of 0.71 mm polypropylene mesh. This material was chosen because polypropylene is fairly resistant to acidic conditions and the mesh size is small enough to prevent the material falling out and excludes macrofauna. One weighed portion of ~ 0.5 g (0.3-0.9 g) of air-dried sample was transferred to every litterbag. The four replicate litter bags were buried back to the same plot from which the material was taken at Whim in April 2007 and at the other sites in July/August 2007; at Moor House and Cairngorms study sites only control material was collected and used as there was no N addition at these sites. The litter bags were placed 5-10 cm below the surface *i.e.* to the similar depth as the samples had been taken.

At Whim one litter bag was removed from each plot every 6 months (October 2007, April 2008, October 2008 and April 2009), and at the other sites one bag was

sampled every time that the site was visited (four times in 16 months at Moor House and three times in 24 months at Cairngorms, snow cover excluded more frequent sampling in Cairngorms). In the laboratory the samples were very carefully cleaned to remove extraneous material *i.e.* litter that had fallen in, moss penetration and fine roots of vascular plants. Mass loss from the samples was established by air-drying to constant weight. The sample was then oven-dried, ground with a ball mill and analyzed for C and N as with the first samples.

At Whim rhizon samplers were placed alongside the litter bags to collect water samples from the moss layer at the same depth where the litter bags were incubated, *i.e.* 5-10 cm below the moss surface. Rhizon samplers (Rhizon, Eijkelkam, B.V., The Netherlands) are plastic tubes that have a semi permeable membrane, made of a 0.45 micron filter, that mimics plant roots and collects water and nutrients from the surrounding pore space. A syringe was used to apply suction to the tube, drawing water and nutrients through the filter into the syringe over a period of days. Water samples from the syringes were collected twice a month if possible, but at least once every month, starting in July 2007 and continuing until November 2008. Because the amount of water in the syringe depended on the amount of rain and the ability of the hummock to retain moisture it was not always possible to obtain sufficient sample from each plot for all of the different analyses, so every variable was not measured for each date. The sample was divided in two and a sub sample used for measuring DOC was frozen immediately until analysis. The other half was further divided with one part used for measuring pH (Mettler Toledo MP220 pH meter) and the other filtered and then frozen. DOC was analysed using a DC-80 Total Organic Carbon (TOC) analyser (Rosemount-Dohrmann) using a sub sample of 200 µl and total N, NH_4^+ and $\text{NO}_3^-/\text{NO}_2^-$ were measured by continuous flow analyser (San ++ Continuous flow analyser, Skalar analytical BV, the Netherlands). Data corresponding to the first 9 months of the study were lost through numerous technical problems with the instruments, which meant the defrosted samples no longer provided a reliable indicator of the different N forms.

To get an estimate for the moisture level of *Sphagnum* twice a month at the same time when the dip wells were measured and the water samples from the rhizon samplers taken a small sample (less than 10 individuals) of *Sphagnum* was taken from every study plot (from the same location where the litter bags were incubated). These samples were transported in plastic bags to the laboratory as quickly as possible and they were kept in the fridge until handled. As soon as possible after returning to the laboratory between 1 and 6 individuals from every sample was/were chosen, the first three cm cut off and weighed instantly to get the fresh weight. After this the individuals were dried in the oven at 70°C at least for 72 hours after which they were weighed again to get the dry weight. A fresh weight/dry weight ratio was calculated for every plot using the total weight of all the individuals.

Statistical analysis

The PK addition was treated as a separate study: the effects of N addition, N form N dose and interactions were tested separately with and without PK. All the data was tested for normality and homogeneity and if needed, \log_{10} transformed. ANOVA was used for testing N addition, N form, N dose, and PK addition, N dose with PK and N form with PK effects on %N in the decomposing material during 18 months and % mass loss during 18 months. REML variance component analysis for repeated measurements was used for analysing the effects of N addition, N form, N dose, and PK addition, N dose with PK and N form with PK on pore water DOC.

Results

Rainfall and water table

Both years 2007 and 2008 were very wet with above normal rainfall. The total rainfall was 1235 mm in 2007 and 1400 mm in 2008. The wetness of the hummocks *per se* was not measured, but monthly rainfall exceeded 50 mm in all months except May 2008, with no prolonged dry spells. The mean water tables at the study site for

each month are presented in Table 5.2. The trend of fresh weight/dry weight ratio between September 2007 and August 2008 is presented in Fig. 5.1.

Table 5.2. The mean water table depth (cm below ground level) for the whole study site for 2008.

Month	Water table depth (cm)
January	0.37
February	5.34
March	2.25
April	3.64
May	13.83
June	11.77
July	10.84
August	3.21
September	4.73
October	1.80
November	1.47
December	3.41

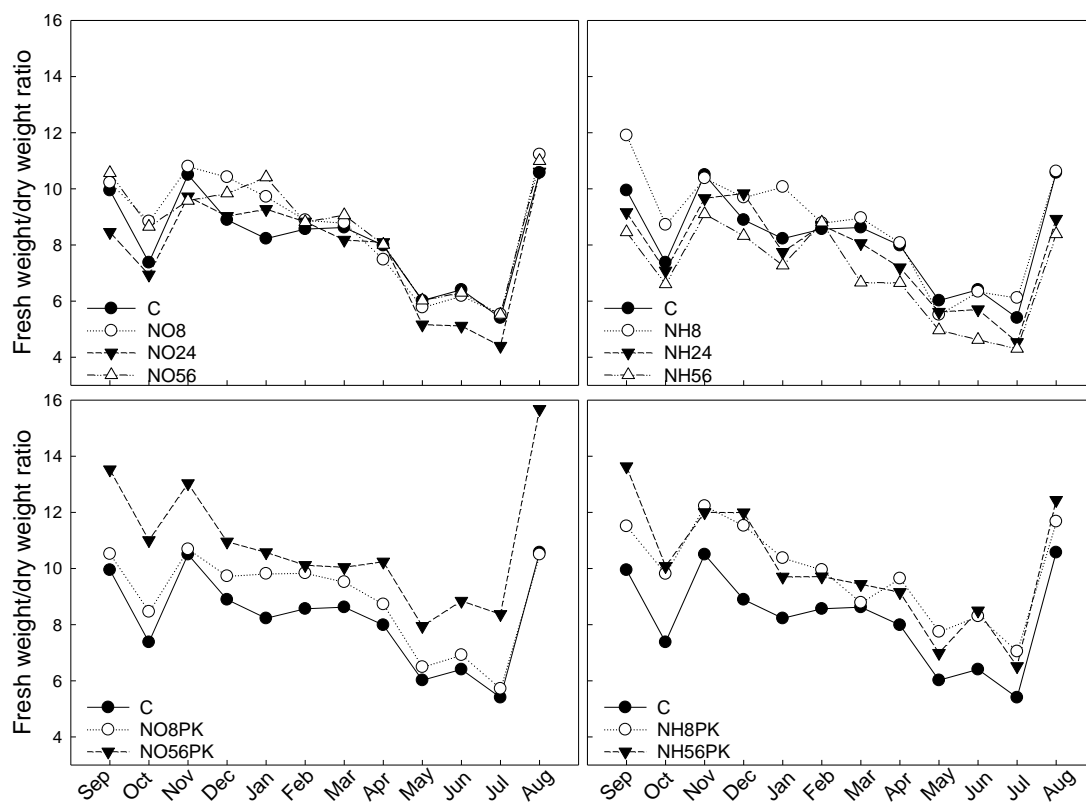


Figure 5.1. The trend of fresh weight/dry weight ratio of *Sphagnum* taken from the hummocks between September 2007 and August 2008.

Litter bags

Although fine roots that had grown inside the bags and litter that had clearly fallen into the bag during the incubation were removed, in some cases there was no weight loss but the weight had increased during incubation; in the last set of litter bags that was taken up after 24 months many of the bags recorded higher weights. This can be a common problem with bags the longer they are left out (see Rochefort *et al.* 1990; Lang *et al.* 2009 with *S. fuscum*). All the data are presented in Fig. 5.2; however due to the extent of this problem in the litter bags incubated for 24 months only the data for the first 18 months have been used in the statistical analysis.

The results could have been affected by some factors other than N deposition. The structure of the hummock can affect the decomposition rates as in higher hummocks the litter is being incubated in more oxidized conditions. It is also possible that different bags were in closer contact with the surrounding *Sphagnum* than others and therefore decomposed at different rates. However, these differences are not expected to be large between the different plots and treatments and therefore the results should reflect real differences in the decomposition rates. The decomposition, measured as mass loss after 12 months ranged between 3% and 22% and after 24 months between 1% and 27% in all the plots (Fig. 5.2, Table 5.3). Without two exceptions the treatment ranking remained the same throughout the duration, with all the N addition treatments having a slower decomposition rate than the control, *i.e.* N addition decreased mass loss ($P=0.026$ after 6 months, $P=0.002$ after 12 months and $P=0.062$ after 18 months). This was also true for the 24 month measurement set. Neither N dose nor N form significantly affected mass loss rate after 6, 12 or 18 months. Effects of adding PK on decomposition became significant after 18 months when NPK increased the decomposition rate compared to 'N only' treatments ($P=0.07$).

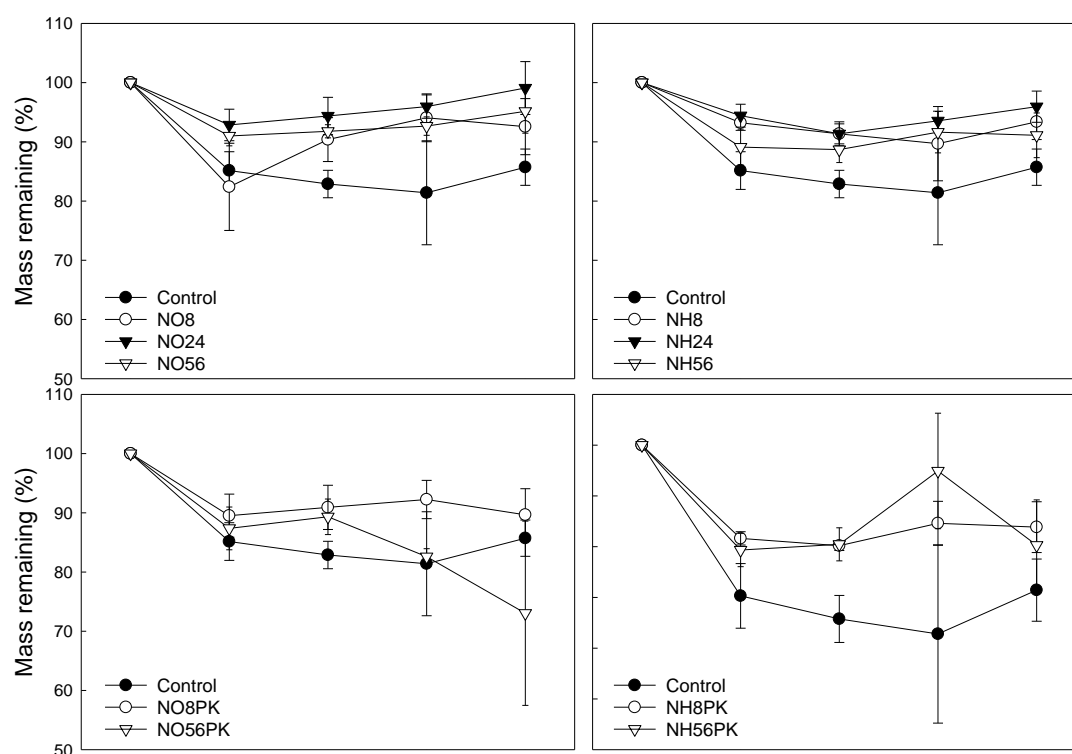


Figure 5.2. Mass remaining (%) \pm SE in the different treatments after 6, 12, 18 and 24 months. The trend was the same in all the measurement sets, *i.e.* N addition decreased decomposition but there was no form or dose effect. PK additions increased mass loss compared to N only treatments, but only after 18 months.

Table 5.3. Remaining mass (%) in different treatments after 12 months incubation.

Treatment	Mass left (%)	+PK	Mass left (%)
C	83		
NO8	90	NO8PK	91
NO24	94		
NO56	92	NO56PK	89
NH8	91	NH8PK	90
NH24	91		
NH56	89	NH56PK	90

The material used for the litter bags at the beginning of the experiment and all the material from the litter bags after incubation were analysed for %N and %C.

However, due to problems with the analysis of the last two samplings only the results from the first 12 months are presented. The complete data set was used in the analysis because treatment comparisons were not affected by the different analytical

methods. The material that went into the litter bags from the treatments was significantly different with respect to N content: both N addition ($P=0.005$) and N dose ($P=0.035$) increased %N (Fig. 5.3). After six and 12 months the effects of N dose could still be seen as higher %N in the decomposing material ($P=0.0026$ and $P=0.0024$). After 18 months this effect had disappeared, i.e. all the treatments had similar %N. Whether the N was added as oxidised or reduced N made no significant difference. At the start of the experiment %N was lower in material treated with PK ($P=0.008$) compared to N only treatments and this effect was still present after 12 months ($P=0.034$), but again had disappeared after 18 months (PK effect was not significant at 6 months). Increasing N dose with PK also significantly increased %N in the decomposing *Sphagnum*, in the beginning ($P=0.019$), after 6 months ($P<0.001$) and after 12 months ($P<0.001$) but not beyond although as can be seen from figure 5.3 this is mainly due to NOPK and not NHPK treatments.

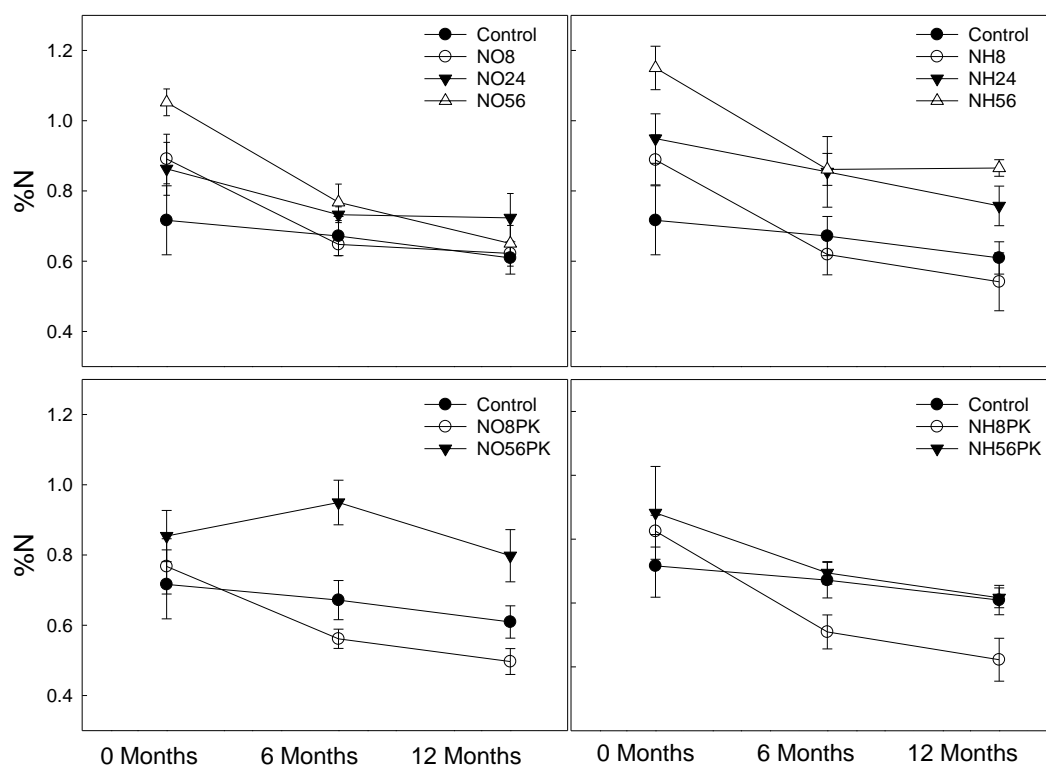


Figure 5.3. The %N \pm SE in the stem parts that were used in the decomposition litter bags before incubation, and after 6 months and 12 months of incubation.

The concentration of C stayed the same throughout the 24 months and therefore C:N ratio followed the pattern of %N (Fig. 5.4). The percentage of N lost during incubation correlated well with the initial amount of N, i.e. the lower the C:N ratio in the beginning the faster N was lost (Fig. 5.5).

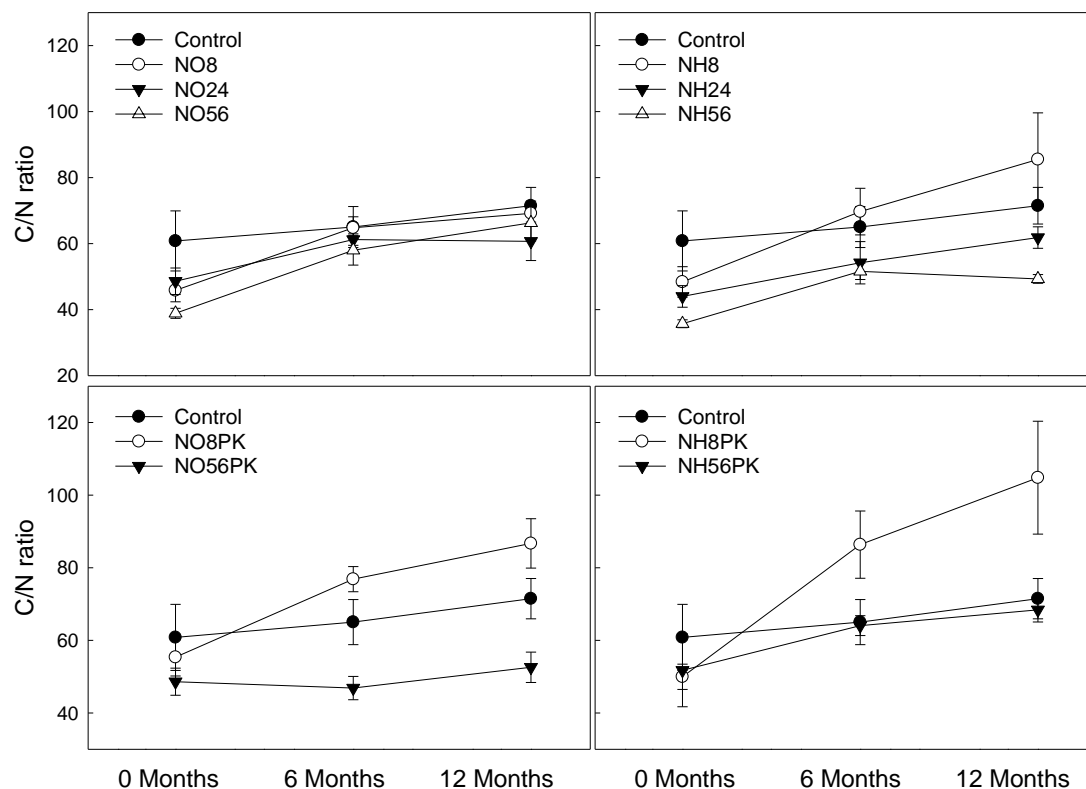


Figure 5.4. The C/N ratio \pm SE in the stem parts that were used in the decomposition litter bags before incubation, and after 6 months and 12 months of incubation.

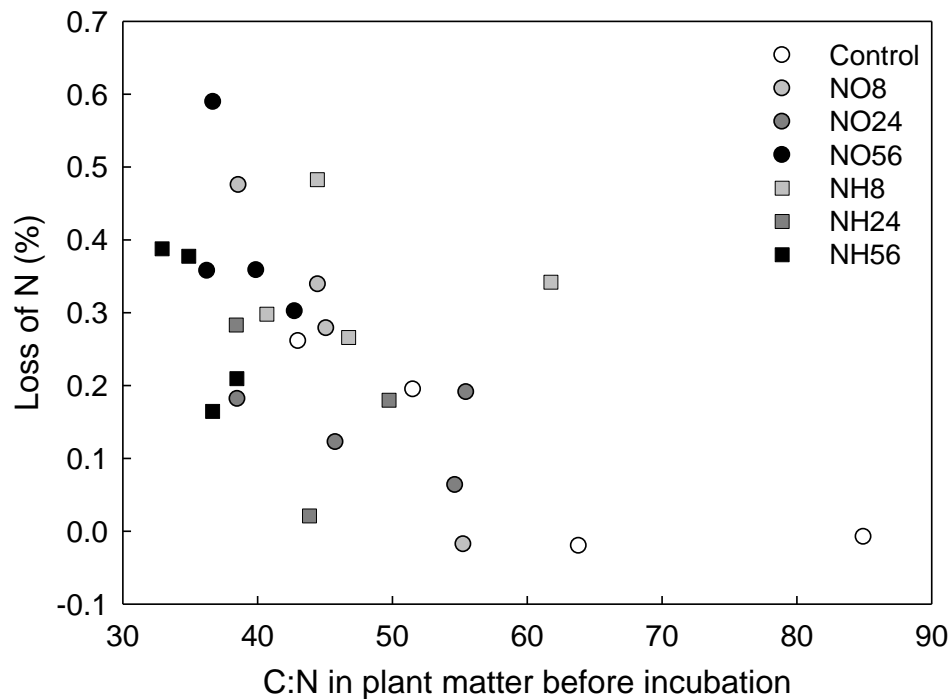


Figure 5.5. The C:N ratio at the beginning of the experiment before incubation. Negative change in %N means increased N in the plant material during incubation.

At the Cairngorms site the mass remaining after nine, 13 and 25 months was always ~95% so it seems that at this site decomposition is very slow (Table 5.4). This is consistent with the short summer and low temperatures. When Whim bog was compared with Moor House that has more similar temperatures and length of snow free time, a very small difference in decay rates was found. After 12 months the mass remaining at Whim control plots was 83% compared with 85 % at Moor house. At Whim and Moor House a clear decrease in %N and simultaneous increase in C:N ratio was found but at Cairngorms the trend was not very clear. Increase in the %N at Cairngorms after 12 months is probably due to measurement error, rather than an actual increase. The C:N ratio increased faster at Moor House and Cairngorms but this was not reflected as a higher decomposition rate at either site.

Table 5.4. Mass remaining \pm SE in the litter bags after 6 and 12 months and %N \pm SE and C/N ratio \pm SE of the litter bag material in the beginning of the experiment and after 6 and 12 months at all three study sites (results for Whim are for the control plots). (Moorhouse mass loss values are within the 20% range observed for mass loss over 12 months by Clymo and Reddaway 1972).

Time (months)	Mass remaining (%)			%N		C:N ratio		
	6	12	0	6	12	0	6	12
Whim	85 \pm 3.2	83 \pm 2.3	0.72 \pm 0.1	0.67 \pm 0.006	0.61 \pm 0.005	61 \pm 9	65 \pm 6	71 \pm 6
Moor House	93	85 \pm 5.7	0.53 \pm 0.1	0.48 \pm 0.008	0.47 \pm 0.1	80 \pm 16	98 \pm 19	111 \pm 28
Cairngorms	95 \pm 0.1	95 \pm 1.1	0.19 \pm 0.002	0.12 \pm 0.008	0.32 \pm 0.002	220 \pm 17	360 \pm 26	147 \pm 8

DOC peaked in all the treatments in the middle of the summer (Fig. 5.6). Although in the summer time DOC in the control treatments was higher than in the 'N only' treatments, no significant effect of N addition, N form or N dose was found. Adding PK did not affect DOC compared to 'N only' but in the PK treatments there was a form response ($P=0.022$), i.e. in the NOPK treatment DOC increased more than in the NHPK treatments, which appeared to reduce DOC (Table 5.5) (Fig. 5.6). Also PK*form ($P=0.002$) interaction was found. These interactions confound the interpretation of the dose and form effects, but show that when nutrient limitation is removed, nitrate causes the opposite effect to ammonium, stimulating leakage of DOC into the hummock by 104, 34, 31 and 66% over the different seasons (Table 5.5).

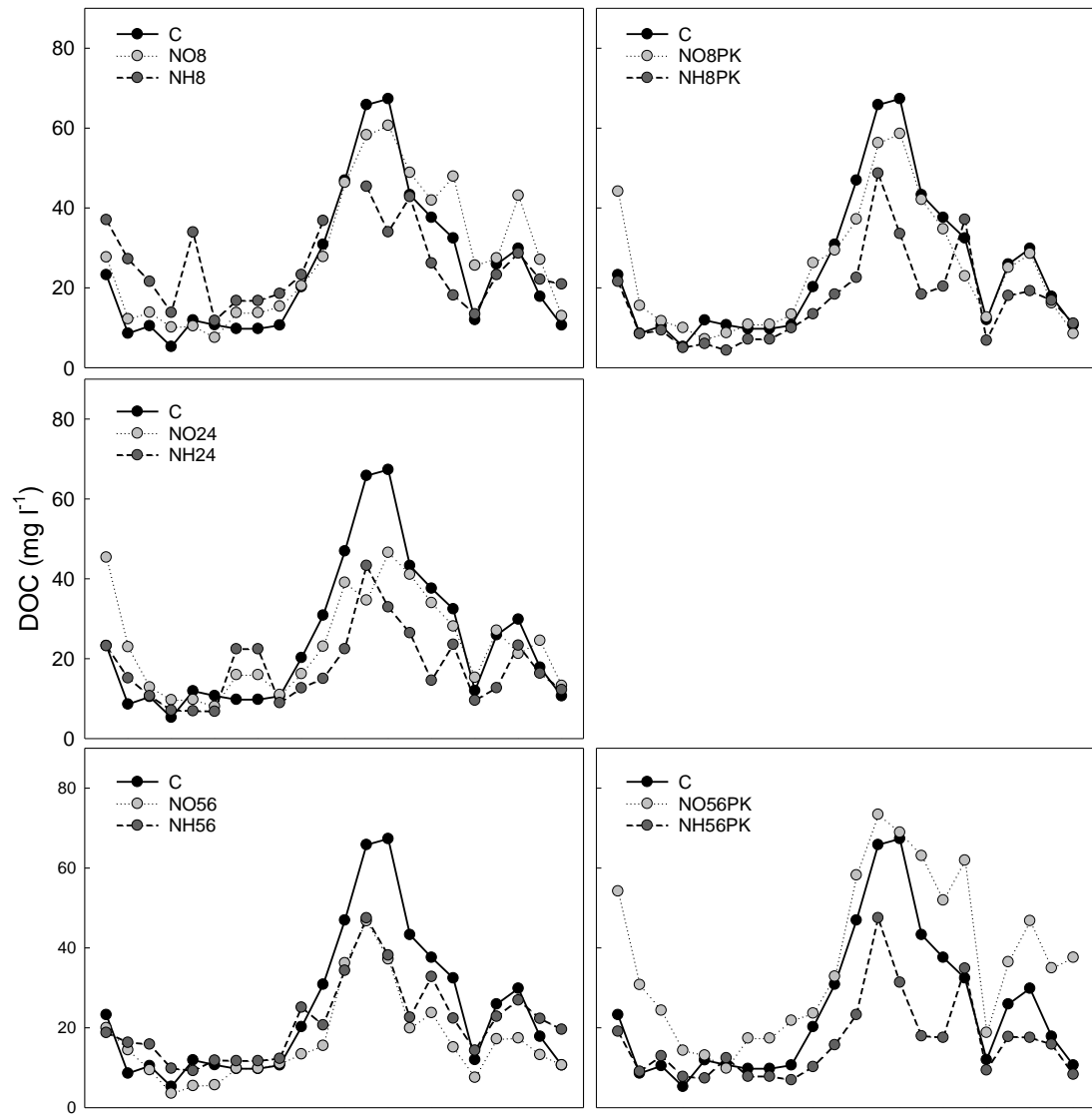


Figure 5.6. The measured DOC at the different treatments between November 2007 and November 2008. The pore water was collected with rhizon samplers between the litter bags at each study plot.

Table 5.5. Seasonal averages in DOC (mg l^{-1}) for the different treatments between November 2007 and November 2008.

	Nov -Feb	Mar-May	Jun-Aug	Sep-Nov
C	11.7	21.3	43.1	23.2
NO8	13.6	22.9	47.2	30.6
NO24	18.1	20.2	33.3	22.4
NO56	9.8	16.0	25.0	15.2
NH8	24.2	20.1	30.0	23.0
NH24	11.6	17.3	25.0	16.5
NH56	13.6	19.3	29.6	22.6
NO8PK	16.2	21.3	37.9	21.5
NO56PK	24.4	28.5	56.3	38.5
NH8PK	9.1	13.1	27.5	16.6
NH56PK	11.4	11.9	26.4	15.9

As expected, nitrate increased the water pH and ammonium decreased it (Fig. 5.7).

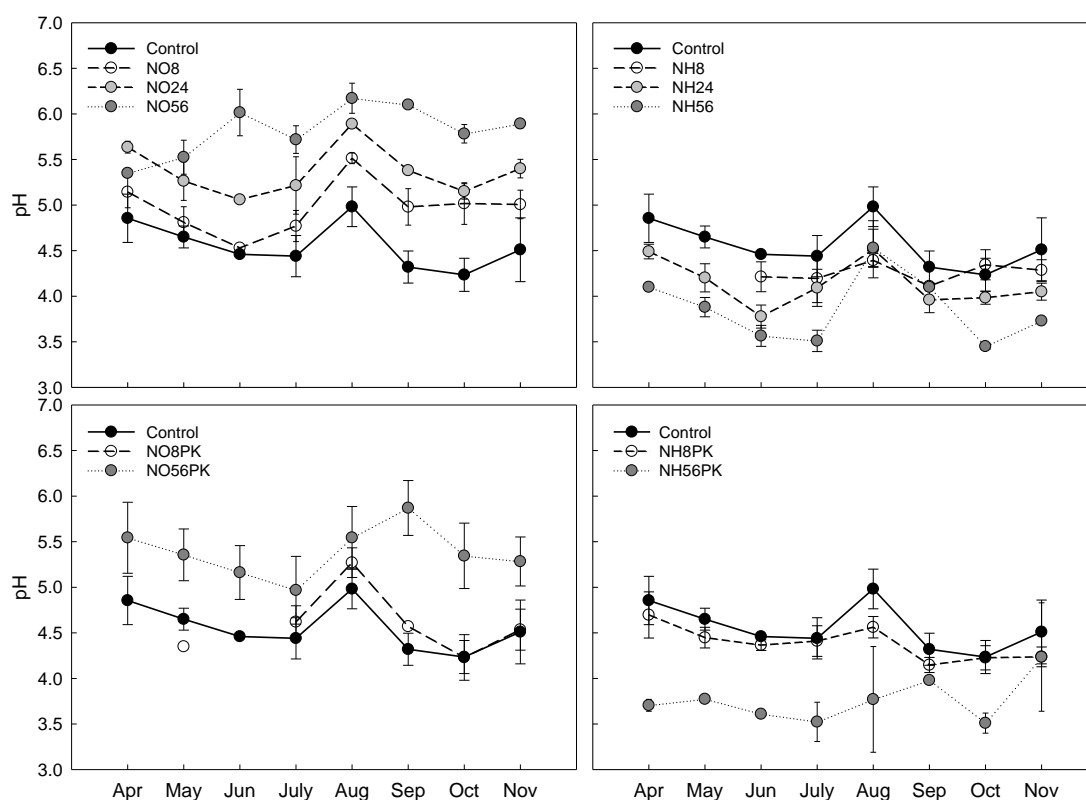


Figure 5.7. pH \pm SE measured from the pore water collected with rhizon samplers between April 2008 and November 2008. The value is monthly average \pm SE for every treatment. Where the SE is missing there was only one measured pH value for that treatment during that month.

Total N in the pore water increased in the high N treatments especially in the NO56 treatment (Fig. 5.8). Organic N values are plotted in Fig. 5.9 and it can be seen from the y axis that the increase in total N in the high N treatments was due to an increase in organic N. In all other treatments except NO56 the amount of total N was only slightly higher than organic N (Fig. 5.9 & 5.10). Adding PK had little effect on the amount of N found in the pore water.

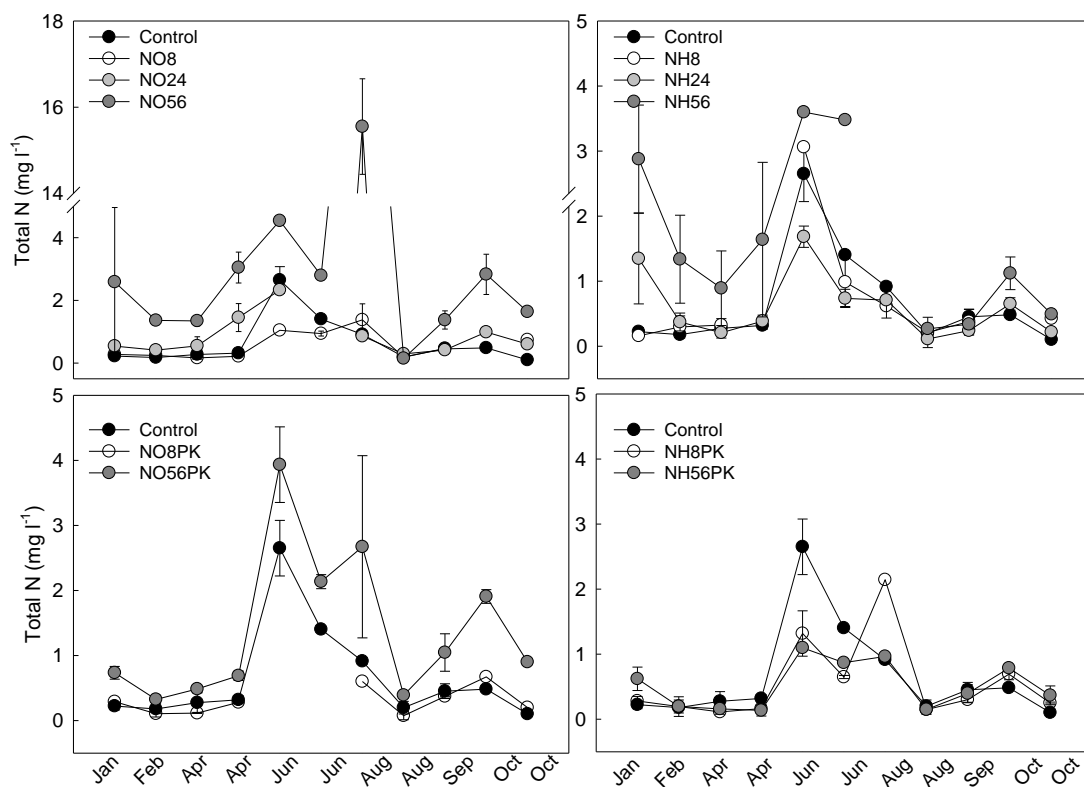


Figure 5.8. The amount of total N measured in pore water collected with rhizon samplers between January 2008 and November 2008. The value is monthly average \pm SE for every treatment. In the case of SE missing there was only one measured value for that treatment during that month.

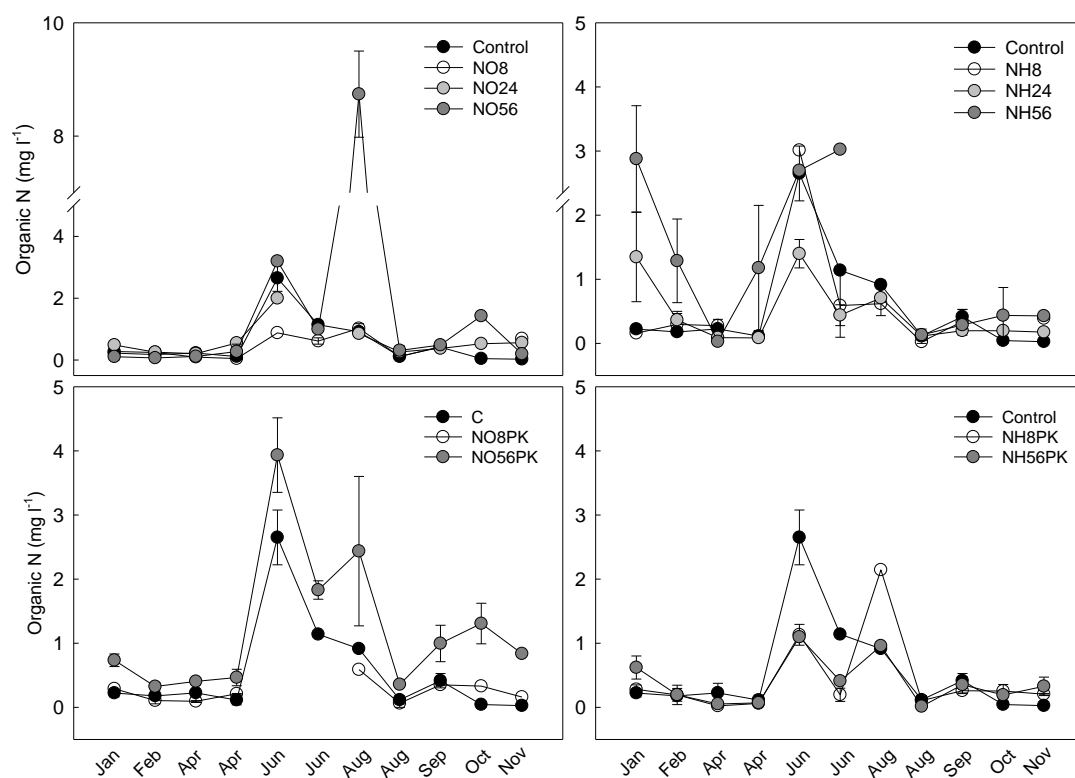


Figure 5.9. The amount of organic N measured in pore water collected with rhizon samplers between January 2008 and November 2008. The value is monthly average \pm SE for every treatment. In the case of SE missing there was only one measured pH value for that treatment during that month.

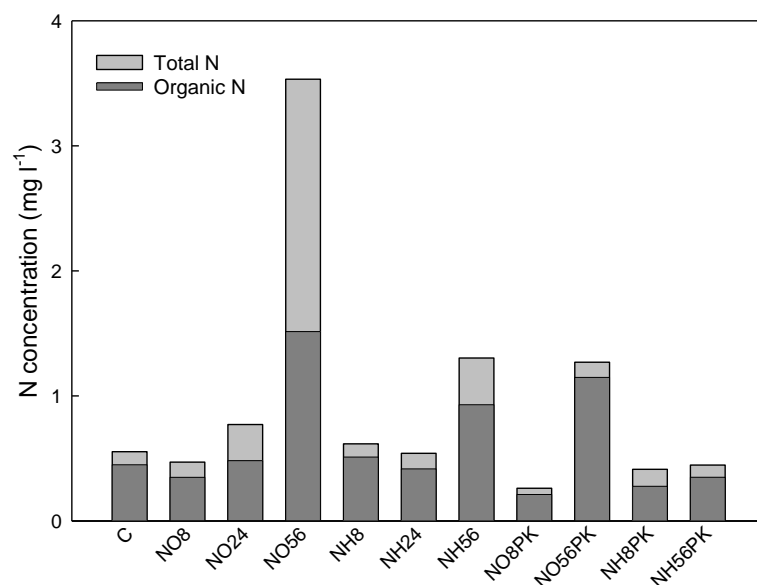


Figure 5.10. Average amount of total N and organic N in the pore water collected with rhizon samplers in the different treatments during 2008. Most N was in organic forms except in the NO56 treatment where most N was in inorganic form.

Discussion

This study followed mass loss from *Sphagnum* that had been treated with N for 5 y prior to setting up the litterbags. The material was taken 3-8 cm below the capitulum and placed in bags which were subsequently replaced back within the hummocks. Several other decomposition studies have used a similar technique, taking stem parts of *Sphagnum* from a similar position, ~2 - 5cm below the capitulum (Bubier *et al.* 2007; Limpens and Berendse 2003b). The advantage of this technique is that it looks at decomposition *in situ*, *i.e.* under field conditions, which means the findings should be more appropriate for predictive modelling of the response of *Sphagnum* decomposition to elevated N deposition. An alternative approach, quite widely used, is to remove *Sphagnum* from the field, return it to the laboratory and keep it moist in containers suited to measuring gas exchange, then after varying incubation lengths measure the amount of CO₂ released as a surrogate for C mineralised (Hajek *et al.* 2010; Bragazza *et al.* 2006; Aerts *et al.* 2001).

Does enhanced N deposition affect rates of S. capillifolium deposition?

The only other study, that we are aware of, to have examined the fate of *S. capillifolium* in litterbags is the study of Bubier *et al.* (2007) in the bog at Mer Bleue in Canada. Although the study by Bubier *et al.* was only done with the subspecies *capillifolium* and the present study with both subspecies *capillifolium* and *rubellum* and the research was carried out on a different continent, it still represents the closest comparable study to the present one. Differences in the ecology and response to increased N between the two subspecies are also assumed to be small. This study also used very similar levels of N deposition to Whim (max. 60 kg ha⁻¹y⁻¹ as NH₄NO₃), included PK treatments and had only a slightly higher ambient deposition. Litterbags containing stems taken from below the capitulum were incubated *in situ* for 5 years but showed no significant effects of N addition with or without PK. At Whim the N addition (all N treatments compared with control) significantly decreased mass loss, with the effect declining over time so that after 18 months the probability of N affecting mass loss was no longer significant. Also as found at Mer

Bleue, adding PK had no effect on decomposition. Interestingly, at Whim there were positive effects of NPK observed on the extension growth of *Sphagnum* (Chapter 4) which suggests that *Sphagnum* was co-limited by P and possibly K. An earlier study at Whim over the first few years of treatment also implied co-limitation (Carfrae *et al.* 2007). Aerts *et al.* (2001) and Hoosbeek *et al.* (2002) suggested that when these nutrients were co-limiting effects of N deposition on decomposition would be minimal which is in accordance with the results from this study where NPK additions had no effect compared to 'N only'. What is clear from both the Mer Bleue and Whim study together with the observations from Moorhouse and Cairngorms is that *S. capillifolium*, a hummock former, decays only very slowly and in the short-term, up to 5 years, this slow rate of mass loss is not increased by enhanced N deposition, even when the availability of PK is increased.

As indicated earlier results of *Sphagnum* decomposition studies with N have been contradictory: Coulson and Butterfield (1978) examined the effect of 100 kg ha⁻¹y⁻¹ added as a dry fertiliser and saw a significant 20% reduction in mass loss over 18 months from *S. recurvum* litter. This experiment used two annual dry fertiliser applications prior to collecting the material for the litterbag study and commented how the N content had declined over time. The study also differed from Whim in that decomposition was investigated under ambient N conditions *i.e.* the litterbags were put into an unfertilised part of the bog. Interpreting the effect of the added N in that study, in terms of understanding the effects of chronic long-term N deposition must therefore be questioned. Verhoeven *et al.* (1996) compared decomposition in a polluted and unpolluted mire but used cotton strips rather than looking at mass loss. Aerts *et al.* (2001) used two quite low N additions (5 and 10 kg N ha⁻¹y⁻¹) over several years and evaluated decomposition as a potential based on CO₂ evolution. They concluded that high N litter decomposes faster than low N litter, although CO₂ levels were not significantly affected. Limpens and Berendse (2003b) took litter of *S. magellanicum* from a mesocosm study that had received 50 kg N ha⁻¹y⁻¹ for 3 years and placed the bags in the field for one year and found a 30% increase in mass loss from N addition. Thus the evidence for realistic N additions increasing decomposition is far from convincing and except for the latter study, much of what

has been written is based on inference, with a scarcity of *in-situ* data. Obtaining good data remains a challenge; the longer litter bags are left out the greater the likelihood of experimental artefacts *e.g.* material getting into the bag. The question also remains, when comparing studies, of whether the material was in the same state of decay before it went into the litterbag *i.e.* how much had already been lost. Furthermore in the absence of data on the chemistry of the litter remaining it is not possible to predict how decomposition will subsequently be affected and what the implications are for mineralisation.

There is a consensus view emerging from the study of forest litter (Berg and McLaugherty 1987) that N additions speed up the initial decomposition process but slows down decomposition of the more recalcitrant litter. Berg *et al.* (1982) found that the initially fast decomposition of N-rich pine needles slowed down after 500 days and that after three years the mass loss rate for both N-rich and N-poor needles was the same. They too found a tendency for N to reduce mass loss after three years. High phenol content can form complexes and retard decomposition (Hättenschwiler and Vitousek 2000). However, Bragazza & Freeman (2007) found that increased N deposition decreased the amount of polyphenols in *Sphagnum* which should increase decomposition rate. Some explanation of this conundrum comes from a recent study by Hajek *et al.* (2010) who using a stepwise approach to examine compounds believed to slow-down decomposition showed that in *S. magellanicum* removal of polymeric lignin like phenolics had little effect on C mineralisation. Instead, the cell wall pectin like polysaccharides *e.g.* sphagnum, were the compounds with decay inhibiting properties. According to Hajek *et al.* (2010) *Sphagnum* may affect the activity of the decomposer community by electrostatic immobilisation of extracellular enzymes, which might be seen in an accumulation of dissolved organic nitrogen. The responses of these compounds to N deposition and the implications for decomposition remain to be studied.

The fact that the Whim study and the one at Mer Bleue, using realistic N doses and treatment scenarios, both suggest only small reductions, which became less apparent over time, or no effect of N on decomposition rates give us some confidence in

concluding N deposition has not enhanced the aerobic decomposition of hummock species in areas where the background N deposition is less than twice the Critical Load for ombrotrophic bogs ($5\text{--}10 \text{ kg N ha}^{-1} \text{ y}^{-1}$). However, given that the Whim study showed significant N driven reductions in growth, the amount of *Sphagnum* decomposing will be less. It is possible that in high latitude areas where N deposition is still low, $< 2\text{--}4 \text{ kg N ha}^{-1} \text{ y}^{-1}$, but the systems are not wholly constrained by temperature, N will increase the mass loss of *Sphagnum* due to significant N enrichment of the tissue. Knorr *et al.* (2005) conducted a meta-analysis into the effects of elevated N deposition on decomposition. Their conclusions were based on the premise of relating N deposition to multiples of background deposition. They concluded that when the N load was $> 2\text{--}20$ times background deposition decomposition would be inhibited, only being stimulated at the extreme ends. The results from the present study are in keeping with this.

Are changes in mass loss corroborated by changes in litter chemistry and pore water chemistry?

The litter used in this study had most likely been produced under conditions of elevated N deposition, so not surprisingly the litter from the high N plots had a higher %N than the litter from the control plots. The extra N in the treatment plots compared to the control plots was consumed quickly; the difference between the treatments in %N disappeared after 12 months. Excess N had accumulated as amino acids (van Berg L. unpub. measurements from 2008); these compounds are easily respired/leached and may explain the most rapid mass loss which was seen in the first 6 months. These results concur with those of Limpens & Berendse (2003b) who also found a positive relationship with %N, however, they also observed that the mass loss related to the N:C quotient but that the relationship was only present under certain ratios. The significance of the N content for mass loss appears to be somewhat contentious, and may in part reflect differences in the N concentration in the controls, which provide the benchmark of most comparisons (see also Lang *et al.* 2009).

The pore water was measured within the hummock and will reflect any N that is in the extracellular capillary water of the hummock *i.e.* not just that released from the litterbags. The amount contributed by the litterbags *per se* is unquantifiable. However the measurements do provide some indication of the state of the hummock, whether it is sequestering or leaking N. Pore water measurements available mainly for the second year of the incubations contained approximately 1-4 mg l⁻¹ N and while they were higher in the high N treatments the results weren't significant. This is an important observation because it suggests that a large proportion of the mineral N treatment load was still being retained by the hummock, even with the high N deposition. Also although organic N represented by far the largest fraction of N, the differences between the control and even the highest N treatments were not proportional to the N load. This contradicts the amino acid data which showed a significant effect of N dose and N form. Limpens *et al.* (2003) found similar concentrations of inorganic N (up to 2 mg l⁻¹) in the pore water as in this study, and did see the expected increase in inorganic N, mainly due to ammonium, in their 40 kg and 80 kg N ha⁻¹ y⁻¹ treatments compared to 0 kg N ha⁻¹ y⁻¹ treatment. However, it is difficult to draw any insight into the N pore water chemistry at Whim based on Limpens study.

The pore water pH showed significant treatment effects both with respect to N form and dose, with nitrate increasing pH by almost 2 units with the highest dose and the opposite occurring with ammonium. Given this level of change and the sensitivity of extracellular enzymes and other processes to pH the absence of N form and dose effects on the decomposition is difficult to explain. The activity of the polyphenol oxidase is highly sensitive to pH (Freeman *et al.* 2001), thus the increase in pore water pH seen here might be expected to stimulate an increase in polyphenol breakdown. However, if this occurred it was not detectable as DOC. Interestingly the higher pHs measured with nitrate are approaching those likely to affect the survival of this species (above 5.5 decreases growth considerably) (Clymo and Hayward 1982). How this pH effect is mediated is unclear, but nitrate like ammonium has reduced growth and increased respiration costs (Chapters 4 & 6) and since the start of this experiment the high nitrate N dose, just like the high ammonium treatment,

has reduced the cover (Sheppard *et al.* 2010). DOC likewise was not significantly affected by N form or dose, responding mainly to summer temperatures which were accompanied by increases in DOC. The temperature response supports the view (see Bragazza *et al.* 2006 and references therein) that enzyme activity is the primary controlling influence over DOC concentrations.

Effects of PK additions

In this study P and K were added as K_2HPO_4 in the same ratio as N:P in protein, 14:1 in the spray solution. Both elements are likely to be limiting in these ombrotrophic bogs which rely on deposition and mineralisation to make nutrients available (Lindsay 2010). However, despite increasing the availability of these nutrients decomposition rates were not enhanced as would have been predicted and the mass loss was still below the control. The moderating effect of PK on deposition was also seen in N concentrations in the pore water. There was a form effect with nitrate increasing DOC more than ammonium.

Conclusions

In this *in situ* study with litterbags that contained N enriched litter of *S. capillifolium* our hypotheses were not supported; neither N nor NPK stimulated mass loss and on the contrary they slowed down mass loss, although the effect declined with time. However, the effects were not related to N dose or form. Pore water N concentrations were also insensitive to N dose. pH was affected by N form and this could have affected DOC by improving conditions for the breakdown of polyphenols. However, there was no significant evidence of this. These results are consistent with those measured at Mer Bleue under very similar circumstances where they concluded no effect of N or PK on mass loss after 5 years. While additional N does not appear to enhance mass loss or DOC losses it has been found to reduce productivity leading to less C being sequestered under enhanced N deposition.

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6. Paper III “Long-term enhanced nutrient deposition increases ecosystem respiration and carbon losses from a *Sphagnum* bog”

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Summary

Enhanced nitrogen (N) deposition has increased in the last few decades and caused changes in the functioning of the main peat formers in peatlands, the *Sphagnum* mosses, leading to changes in their CO₂ balance. The aim of this study was to look at the effects of experimental N addition, with and without phosphorus (P) and potassium (K), on CO₂ fluxes from *Sphagnum capillifolium* hummocks in a long-term N addition experiment in the Scottish Borders. For this, static chamber measurements of CO₂ were made during year 2008 on 20 plots (control, and N treatments with 56 kg N ha⁻¹ y⁻¹ of both NO₃⁻ and NH₄⁺ added, with and without PK).

Even in the extremely wet conditions of the study year 2008 almost all the measured fluxes were negative, i.e. *Sphagnum* stands lost CO₂ to the atmosphere, irrespective of the treatments applied. Treatment did not have a significant effect on ecosystem respiration (ER) or net ecosystem productivity (NEP) but adding PK with N increased gross photosynthesis (P_G) significantly compared to the other treatments. Summed monthly averages of net ecosystem productivity for every treatment indicated that increasing N deposition increased the loss of CO₂ from the system. The form of N affected the response: compared to the control adding nitrate increased the CO₂ loss more than ammonium, both with and without PK.

Nitrogen (both forms) exaggerated the stimulatory effect of temperature on ecosystem respiration fluxes, adding PK with N exaggerated the response further. The increasing temperature response of ecosystem respiration with N suggests that in

high N deposition areas the possible increase in ecosystem respiration will be exacerbated with climate change.

Introduction

In the wet and cool conditions of boreal and temperate peatlands, the decomposition of organic matter is expected to be slow. 'Active' peatlands, by definition, sequester carbon (C) because primary production exceeds the rate of decomposition allowing carbon to accumulate as peat (Clymo 1983). In ombrotrophic bogs most of the peat is formed by *Sphagnum* mosses (Gorham 1991). This is because *Sphagnum* mosses create acidic and nutrient poor conditions favouring their own growth at the expense of many other life forms and produce litter that decomposes very slowly (Clymo & Hayward 1982; Van Breemen 1995). It has been estimated that about 455 Gt of C has been stored by boreal peatlands since the last glacial period; this amounts to as much as one third of the global soil C pool (Post *et al.* 1982; Gorham 1991). The current annual accumulation rate in northern peatlands is thought to be 0.07 Gt of C (Clymo *et al.* 1998), although the amount of C sequestered can vary between years, depending on weather conditions (Alm *et al.* 1999; Roulet *et al.* 2007). Consequently, large scale disturbance to peatland ecosystems will have significant repercussions for C balance on a global scale and will potentially have a positive feed-back effect on climate change (Alcamo *et al.* 2007; Moore 2002).

The naturally nutrient poor conditions of ombrotrophic bogs have led to the evolution of mechanisms for high nutrient use efficiency in the constituent species; the species composition and ecosystem processes are naturally well adapted to this low nutrient supply (Van Breemen 1995; Chapin *et al.* 1997). However, in the last few decades N deposition has been increasing in many areas in the northern hemisphere due to anthropogenic activities; industrialization, intense agriculture and fossil fuel burning have led to a three to five-fold increase in N emissions in the last century (Reay *et al.* 2008). Because *Sphagnum* species, like all mosses, take up nutrients directly from the atmosphere (Brown 1982), their nitrogen uptake increases as atmospheric concentrations increase (Pitcairn *et al.* 1995). In recent years,

enhanced N deposition in the field under both natural and manipulated conditions has caused changes both in the productivity and decomposition rates of *Sphagnum* mosses (Rocheffort *et al.* 1990; Berendse *et al.* 2001; Limpens & Berendse 2003a&b; Bragazza *et al.* 2006). The productivity has been found to first increase with low N doses but eventually, with increasing N doses and/or longer exposure periods, to actually decrease (Rocheffort *et al.* 1990; Berendse *et al.* 2001; Limpens & Berendse 2003b). In substrates with a high initial C:N ratio as in peat, decomposition rates in general have been assumed to increase with increasing N (Clymo & Hayward 1982). However, some studies have found an increase but some a decrease in *Sphagnum* decomposition with increased N deposition (Rocheffort *et al.* 1990; Bragazza *et al.* 2006). Besides N, phosphorus (P) and potassium (K) play an important role in peatlands. Often in areas with high atmospheric N deposition, peatlands can be P- or K- limited or co-limited by both nutrients (Aerts *et al.* 1992; Hoosbeek *et al.* 2002). Besides often becoming the limiting nutrient in high N areas, P and its availability can influence the impact of N deposition by enhancing the capacity of *Sphagnum* to assimilate N (Limpens *et al.* 2004).

Nitrogen responses differ between *Sphagnum* species; hummock forming *Sphagnum* species appear to be more vulnerable to increased N than species growing in hollows, perhaps because of their natural position that exposes them to more concentrated deposition and because their higher uptake rate of N (Jauhiainen *et al.* 1998). It has also been found that wetting and drying cycles have a negative effect on *Sphagnum* production (McNeil & Waddington 2003); because hummock species are more likely to experience this, the additional stress might affect their capability to cope with increased N deposition.

Due to the changes in *Sphagnum* productivity and/or decomposition, changes in the CO₂ fluxes are to be expected as well. However, in peatland ecosystems there have been very few studies to examine the effects of added N on CO₂ fluxes, and those few studies have yielded contradictory results. Some have reported small or no effects of fertilization on CO₂ exchange (Keller *et al.* 2005; Gerdol *et al.* 2008) and other studies have found some changes. In an experiment where 30 kg N ha⁻¹ y⁻¹ was

added to a pine bog, both gross photosynthesis (P_G) and ecosystem respiration (ER) were slightly higher in the treatment plots than in control plots but there was no statistically significant difference in the annual CO_2 gas balance (Saarnio *et al.* 2003). Bubier *et al.* (2007) found, in a study on a bog with *Sphagnum* moss, *Polytrichum* moss and vascular plants, that N additions of up to $60 \text{ kg ha}^{-1} \text{ y}^{-1}$ initially raised the rates of maximum P_G compared with the control but after 5 years the treatment reduced the rates, showing how the response to increased N deposition and potential N accumulation is likely to change over time. They also found that the decrease in moss photosynthesis with increasing N additions had a greater effect on the ecosystem P_G than the increase in vascular plant biomass and its photosynthesis, emphasizing the importance of mosses, and particularly *Sphagnum*, for the net CO_2 balance of peatland ecosystems. However, Gerdol *et al.* (2008), in an experiment where up to $30 \text{ kg of N ha}^{-1} \text{ y}^{-1}$ was added to a bog, found that temperature and water table were more important for CO_2 fluxes than N but at the end of the 4-year experiment they found a weak stimulation of CO_2 efflux from the treatment plots.

The above studies have all been made by measuring communities containing both vascular plants and *Sphagnum*. Due to the pivotal role of *Sphagnum* in bog ecosystems and their C accumulation in the northern hemisphere it is important to establish the magnitude of CO_2 fluxes, and their sensitivity to N deposition, in pure stands of *Sphagnum*. It is also important to establish the role of individual species because previous studies have shown that differences in CO_2 fluxes between peatland communities can be large (Waddington & Roulet 2000; Riutta *et al.* 2007). Most of the studies so far have also been relatively short-term and they have underlined the importance of long-term measurements (Saarnio *et al.* 2003; Bubier *et al.* 2007). In addition, previous N addition experiments have not simulated wet N deposition realistically, as N has been added only a few times during the growing season, which might have changed the responses compared to 'real world' N deposition that comes year-round and in small doses. This study addresses some of the shortfalls of previous experiments.

The aims of this study were to: 1) determine the effect of prolonged (six years) increased N deposition on CO₂ fluxes from pure stands of *Sphagnum capillifolium*, 2) study the effect of increasing P and K availability on the effects of N and 3) compare the effects of oxidized N (nitrate) and reduced N (ammonium).

Material and methods

Study site

Whim Moss is an ombrotrophic bog situated c. 30 km south of Edinburgh, Scotland (3°16'W, 55°46'N). It represents a transition between a lowland raised bog and a blanket bog. It is situated 280 meters above sea level and the annual rainfall is c. 900 mm, which is at the dry end of the occurrence of blanket bogs but not atypical for a lowland raised bog. However, 2008 when the measurements were done was an extremely wet year with a total rainfall of 1400 mm. The background N deposition is relatively small compared to many areas in the UK, approximately 8 kg ha⁻¹ y⁻¹ (Sheppard *et al.* 2004). The dominating vascular plants in the area are *Calluna vulgaris*, *Eriophorum vaginatum* and *Erica tetralix*. The field layer consists of several moss species, the most common *Sphagnum* species being *Sphagnum capillifolium* covering up to 80% in certain parts of the bog (Sheppard *et al.* 2004).

The bog is being used for a long term study (2002 to the present) to examine the impact of N deposition by manipulating the N dose in wet deposition and hence to elucidate the effects of different N loads and forms (reduced and oxidized). In the experiment N is added to 44 plots either as nitrate (NaNO₃) or ammonium (NH₄Cl) with doses of 8, 24 and 56 kg N ha⁻¹ y⁻¹ on top of the background deposition. There are also plots with N loads of 16 kg N ha⁻¹ y⁻¹ and 56 kg N ha⁻¹ y⁻¹ where P and K are added with N. The elements P and K are added as K₂HPO₄ with P:N ratio of 1:14. For this experiment only control and 56 kg plots were used. The study area is divided into four blocks and the treatments are randomly assigned inside the blocks, each block containing one plot of every treatment. In every block there is also a control plot which receives only rain water.

The N is added with rain water that is collected with a constructed 178 m² pitched surface and then fed into two 1250-dm³ plastic water tanks. N is added to the rain water and distributed from a central rotating disc at the centre of every study plot. The treatments increase precipitation on the plots by ~10%. The sprayed area exceeds the marked study plots (12.5 m²) with only 50% landing on the study plot. The plots are 12.5 m² and they are 3 m apart to avoid contamination from the adjacent plot. Rainfall, wind speed and direction, solar radiation, net radiation, air and soil temperature (10 and 20 cm below the surface) and water table level are constantly monitored at the site (Sheppard *et al.* 2004).

Field measurements

This investigation focused on *Sphagnum capillifolium* because it was well represented through the plots in the experimental site. Stands with pure *S. capillifolium* were selected to be used for CO₂ measurements (in total 20 plots). The stands were relatively small (diameter c. 10 cm) for two reasons: at the site *S. capillifolium* does not in general form large uniform areas and especially in the high N plots the amount of *S. capillifolium* left after 6 years of treatment preceding this study was not large. Collars made of plastic drain pipe were approximately 15 cm deep with a radius of approximately 10 cm. In July 2007 the collars were inserted into a subjectively chosen spot in all of the 20 plots used for this study. These locations were chosen to be (i) representative of the *S. capillifolium* in the plot in general and (ii) accessible from outside the plot. Measurements were started 3 months after inserting the collars, so that the plants had time to recover from the stress induced. The measurements were made between October 2007 and November 2008, several times a month in different conditions and at different times of day to get as wide range of weather conditions as possible (rainy conditions precluded measurement, due to the likelihood of equipment failure).

Instantaneous net ecosystem CO₂ exchange was measured with a transparent plastic TPX chamber (height 11.2 cm, volume 775 cm³) and a portable infrared gas analyser

(EGM-3 and EGM-4, PP systems, UK), connected to make a closed air circulation. The chamber had a small fan to mix the air, and a deep rubber gasket attached to it to form an airtight seal over the collar. Measurements lasted up to two minutes or until the CO₂ concentration had changed by more than 50 ppm. One or two measurements were undertaken in different fluxes of photosynthetically active radiation (PAR), aiming to create a light response curve by using the natural variation in irradiance. After this the chamber was covered with dark cloth to measure ecosystem dark respiration. The chamber was removed from the collar between the measurements to restore the ambient gas concentration. The EGM recorded the CO₂ concentration, air temperature and PAR every four seconds during the measurement. Soil temperature from 5 cm and 10 cm below the moss surface was measured with a thermometer and water table by a dip well at the same time as the gas exchange measurements were carried out. At the PK plots the dip well was next to the CO₂ chamber but elsewhere the dip well was further away from the collar (anywhere in the same plot); in these cases the height difference of the CO₂ chamber to the dip well was estimated and taken into account when calculating water table values to get a better estimate for the actual water table in the collar.

Data analysis

Hourly fluxes of net ecosystem productivity (NEP) and ecosystem respiration (ER) were calculated from the linear change in gas concentration in the chamber as a function of time. Only data where there was a linear increase in concentration passed the quality test and the remainder were not analysed. An estimation of the gross photosynthesis (P_G) was calculated as the sum of NEP and ER. The data are presented so that negative fluxes represent loss of CO₂ from the vegetation and positive fluxes represent gains of CO₂ for the vegetation. For every month in 2008 when measurements were done (all except January, September and December) a monthly average for all NEP, ER and P_G values was calculated. In this, the hourly flux was multiplied first by 24 and then by the number of days to get the monthly balance. In the calculation it was assumed that there was 12 hours of daylight (NEP flux) and 12 hours night (ER flux) all year round. It was also tested to see if changing

the hours at summer time (three months of four hours of dark) and winter time (three months of four hours of light) would change the results but because the change in the results was very small, it was decided to use 12 hours of light and dark year round. Because no measurements were done in January, September and December, a value for these months was calculated assuming linear change in the fluxes. The cumulative annual CO₂ balance for each treatment was calculated by adding monthly NEP (daytime) and ER (night time) values together.

Statistical testing

The measured values of NEP, P_G and ER were tested with REML variance component analysis for repeated measures to see if there are differences between the treatments. P_G data were plotted against PAR and, if possible, a curve was fitted to the data points. The ER fluxes were plotted against air temperature and an exponential growth curve was fitted. ER fluxes were log_e transformed to facilitate comparison between the responses to air temperature and a regression analysis was carried out using the month of the measurement as a block to take into account the seasonal differences in the measurements. The statistical analyses were carried out using Genstat 10 and Sigmaplot 10.0.

Results

Environmental variables

The year 2008 was wetter than average with 1400 mm of rainfall at the study site (Fig. 6.1). The minimum air temperature during the year was -7.4 °C recorded in February and the maximum air temperature 25.4 °C recorded in July. The peat temperature at 10 cm below the surface followed the air temperature very closely throughout the year.

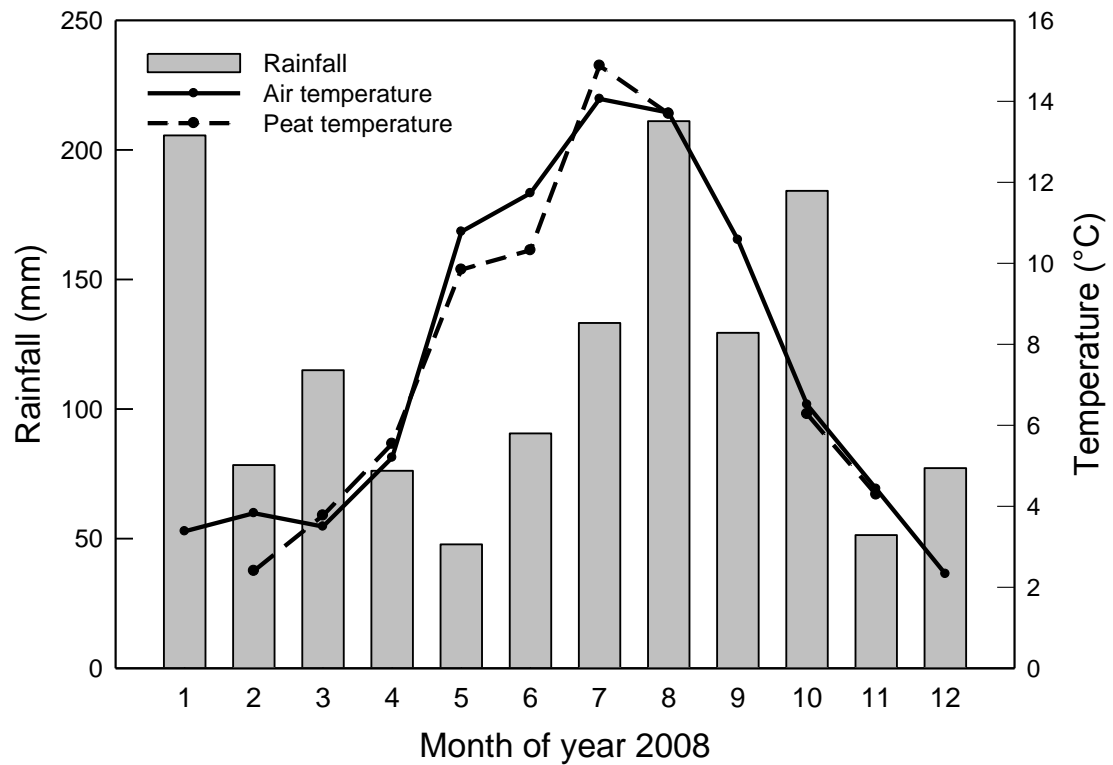


Figure 6.1. The average monthly air temperature, peat temperature at 10cm below surface and total rainfall in year 2008 at Whim bog.

During the year the water table varied in the control plots between -44 cm and the moss surface, and in the treatments between -29 cm and the moss surface (Fig. 6.2). One of the treatments, NO56, was wetter than the other treatments but the differences were very small. In any of the plots the mosses were never totally submerged under water although some of them were regularly very wet.

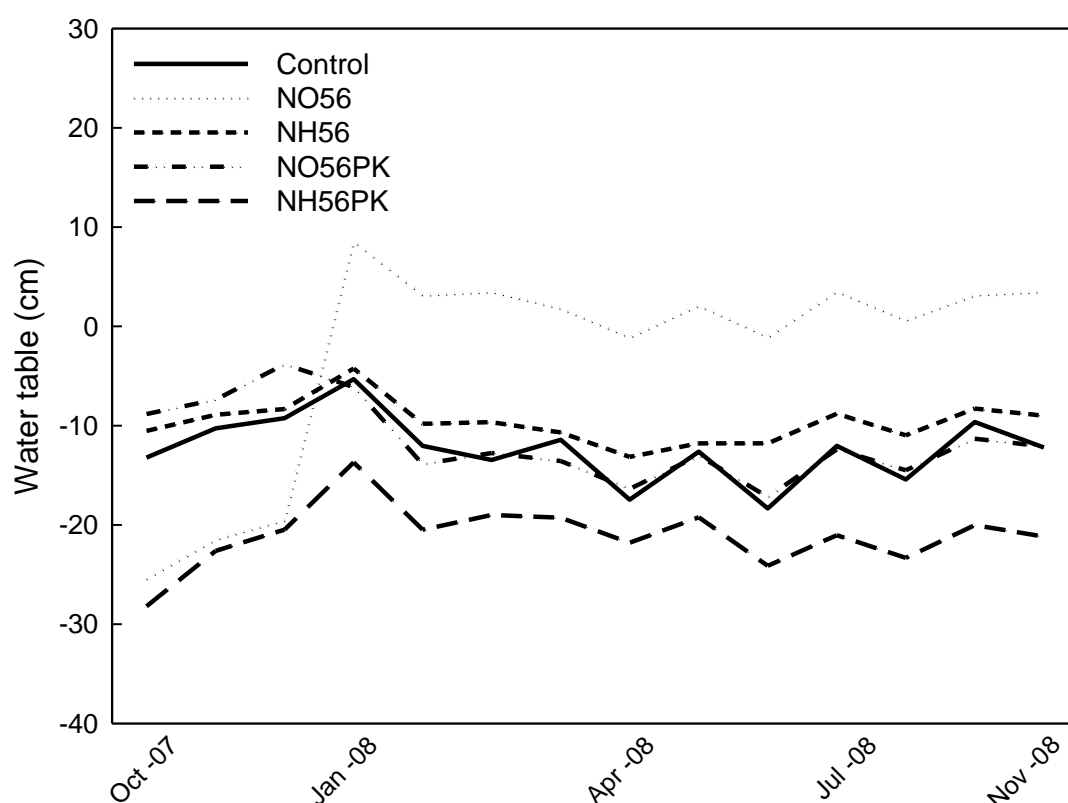


Figure 6.2. The variation in the water table in the different treatments between October 2007 and November 2008 (actual measured value for NPK plots and measured value added with the height difference between the collar and dipwell for control and ‘N only’). The gaps between actual measurements were filled by assuming linear change. The represented values are averages of four study plots for each treatment.

Measured fluxes

Most measured NEP values were negative i.e. the *Sphagnum* stands were losing CO₂ to the atmosphere (Fig. 6.3). All the treatments showed a similar pattern, with highest (most positive) NEP values measured during the spring and autumn and lowest during the summer. The lowest (most negative) ER values were measured during the summer. The measured minimum, maximum and mean values for NEP and ER are shown in Table 6.1.

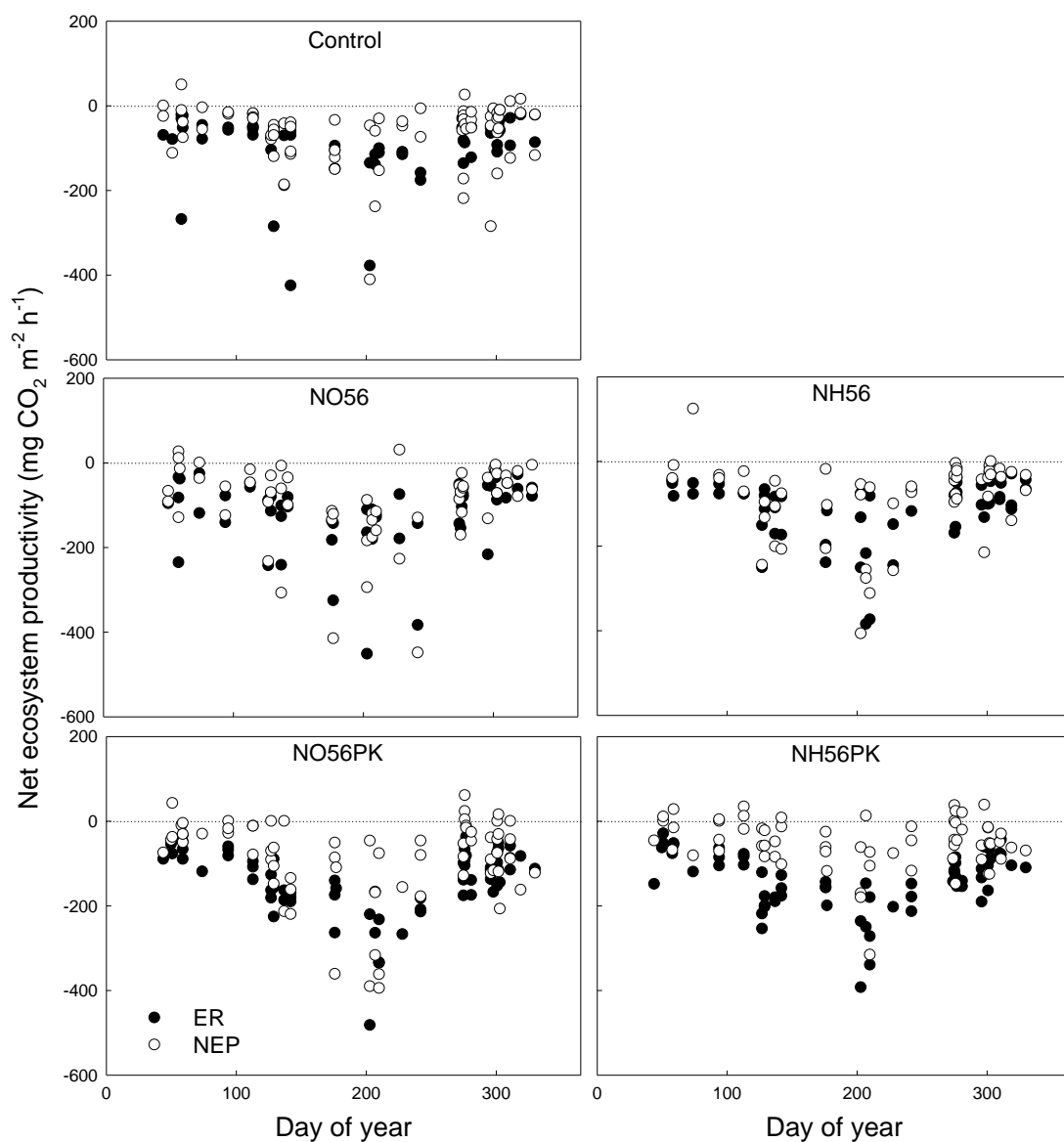


Figure 6.3. Measured CO₂ fluxes from October 2007 to November 2008. Open circles are measured net ecosystem productivity values and closed circles are measured ecosystem respiration values. The sign convention is that negative values represent carbon loss from the vegetation to the atmosphere and positive values represent gain from the atmosphere to the vegetation.

Table 6.1. The minimum, maximum and mean values for the measured net ecosystem productivity (NEP) and total respiration (ER) in $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$.

	NEP _{max}	NEP _{min}	NEP _{mean}	ER _{max}	ER _{min}	ER _{mean}
Control	49	-410	-77	-424	-10	-89
NO56	30	-448	-105	-451	-20	-17
NH56	123	-406	-101	-384	-27	-112
NO56PK	60	-394	-114	-482	-37	-140
NH56PK	38	-315	-95	-392	-29	-140

When monthly averages of NEP, ER and P_G were calculated for every treatment, the pattern during the year became even more obvious (Fig. 6.4). There was no statistically significant effect of treatment on NEP ($P=0.535$) or ER ($P=0.186$). However, this was probably due to large natural variation in the data and both ‘N only’ and NPK treatments seemed to increase ER (especially NPK) and decrease NEP (i.e. it was more negative). A statistically significant increase in the P_G in the NPK treatments compared to control and ‘N only’ was found ($P=0.015$).

When the monthly averages of NEP were summed together to get an accumulation curve of CO_2 during the year (Fig. 6.4d), it was shown that adding nitrate has a larger effect on the loss of CO_2 , than the addition of ammonium. However, the control treatment also lost CO_2 . The estimated loss of CO_2 from the system is presented in Table 6.2.

Table 6.2. The estimated loss of CO_2 from the different treatments

Treatment	Loss of CO_2 ($\text{t ha}^{-1} \text{ y}^{-1}$)	Increase compared to control (%)
Control	6.47	0
NO64	8.08	25
NH64	7.22	12
NO64PK	9.72	50
NH64PK	7.87	22

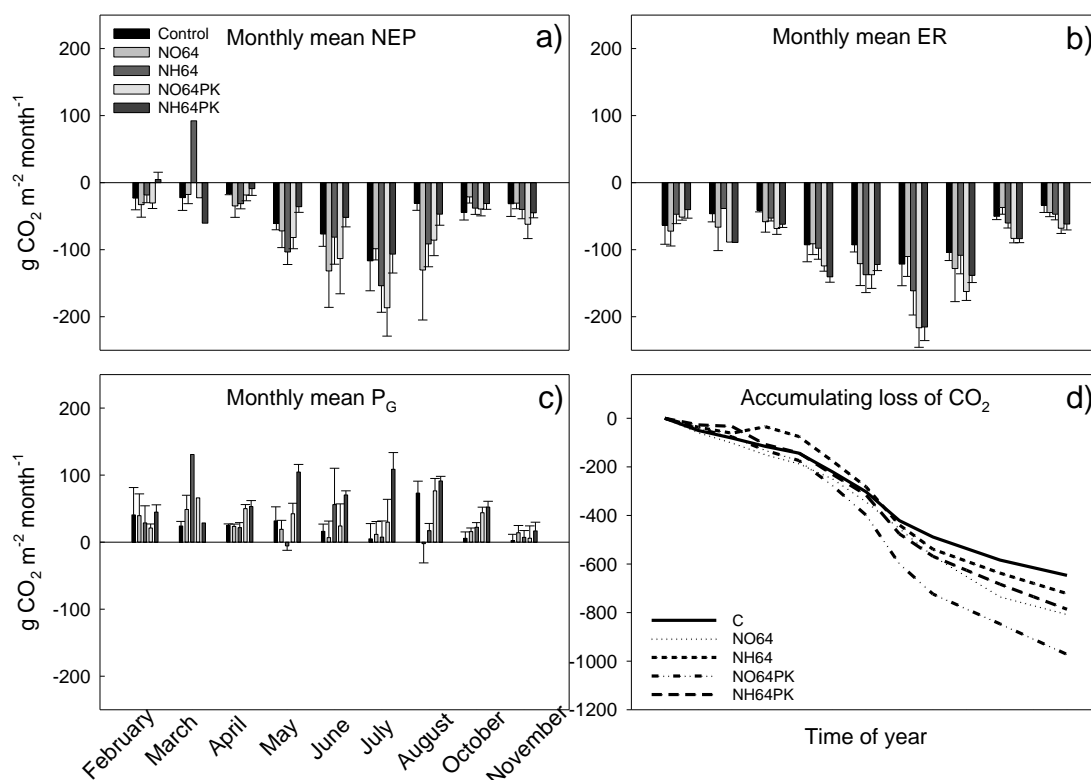


Figure 6.4. a) Monthly mean values for net ecosystem productivity (NEP) , b) ecosystem respiration (ER) and c) gross photosynthesis (P_G) in g CO₂ m⁻² per month \pm SE, missing standard error is due to only one measurement done for that treatment in that month, d) accumulating loss of CO₂ per treatment during 2008 calculated by adding the monthly average NEP and ER values together (values estimated for January, September and December when no measurements were carried out and assuming that there is 12 hours of daylight year round).

Responses of CO₂ fluxes to environmental variables

When the P_G fluxes calculated from NEP and ER measurements were plotted against PAR they showed much scatter. It was not possible to fit any curve to the control and N treatments but for the NPK treatments a curve could be fitted to the data points (Fig. 6.5). However, all the plots except NH56PK seemed to show a similar response to PAR: first with increasing PAR the P_G increased but this turned into a decreasing P_G when PAR reached a high level (around 1000 μ mol m⁻² s⁻¹). In NH56PK the response saturated, but did not show the decrease in P_G with higher radiation levels.

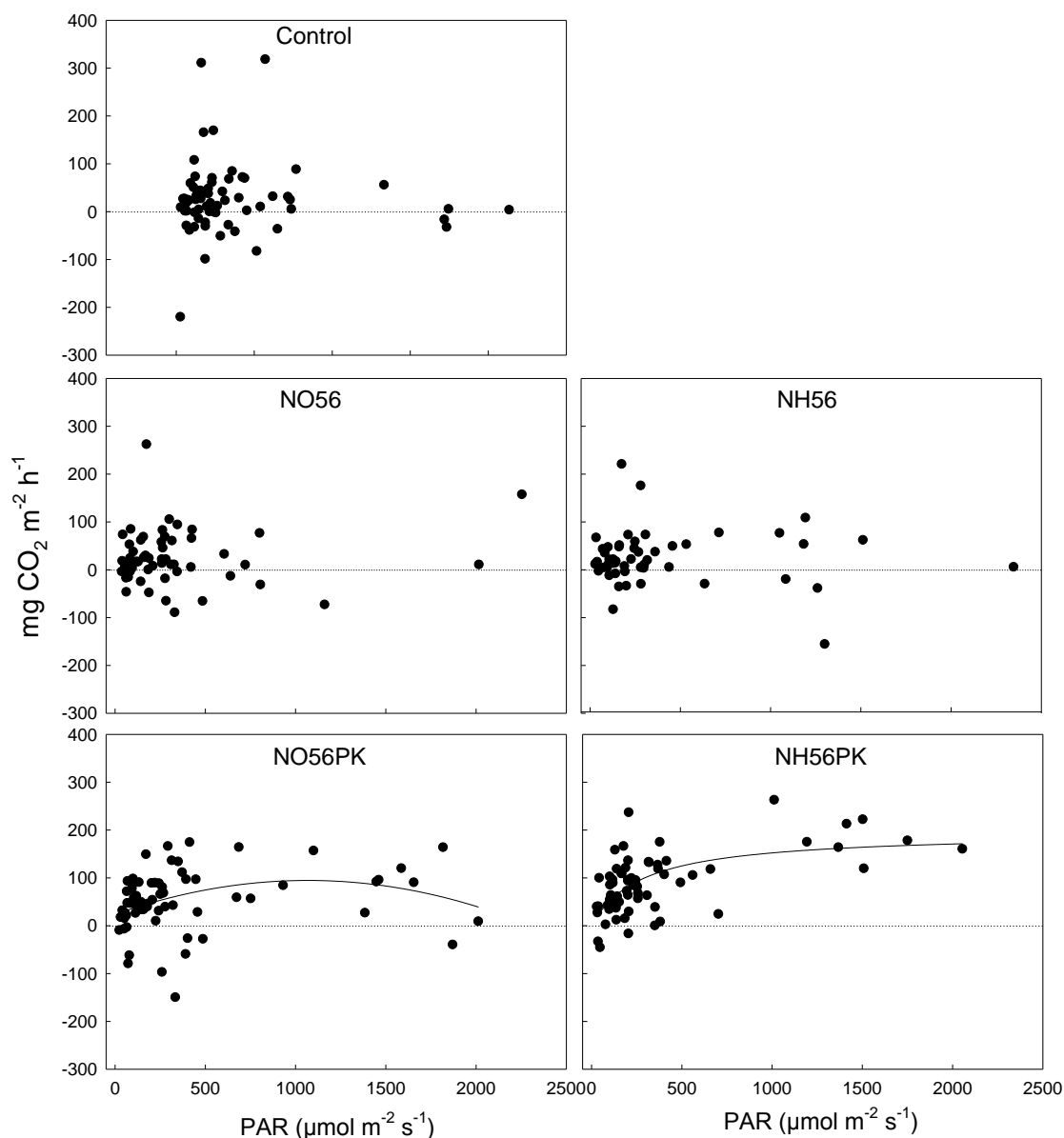


Figure 6.5. Calculated gross photosynthesis values for all the different treatments plotted against photosynthetically active radiation (PAR). It was not possible to fit curves to any other treatments than the NH56PK and NO56PK.

The measured ER fluxes plotted against air temperature showed an increasing response to temperature in the N treatments compared to controls. Adding PK with N increased this response even more (Fig. 6.6). When the ER values were \log_e transformed it was even clearer that the response of respiration increases with air temperature in the N treatments compared to controls ($P < 0.001$) but this was due to the PK treatments ($P = 0.029$ in NH64PK and $P = 0.006$ in NO64PK) (Fig. 6.7, Table

6.3). In addition, this difference increases slightly with increasing temperature, although the difference in the slopes of the lines was not statistically significant.

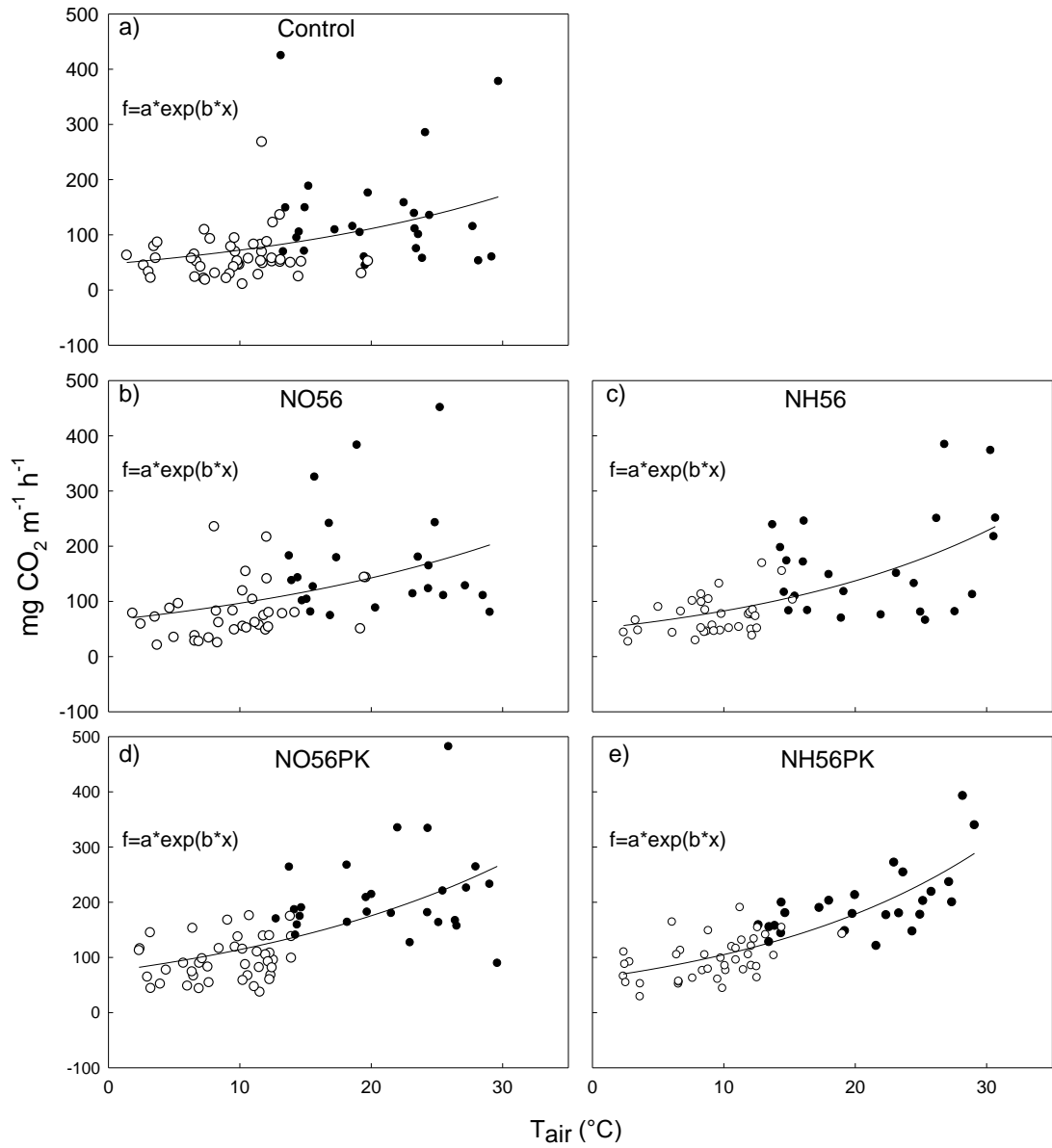


Figure 6.6. Measured ecosystem respiration values plotted against the air temperature inside the chamber during the measurements. Closed circles are measurements during summer time (May- August) and open circles during autumn, winter and spring (October-April).

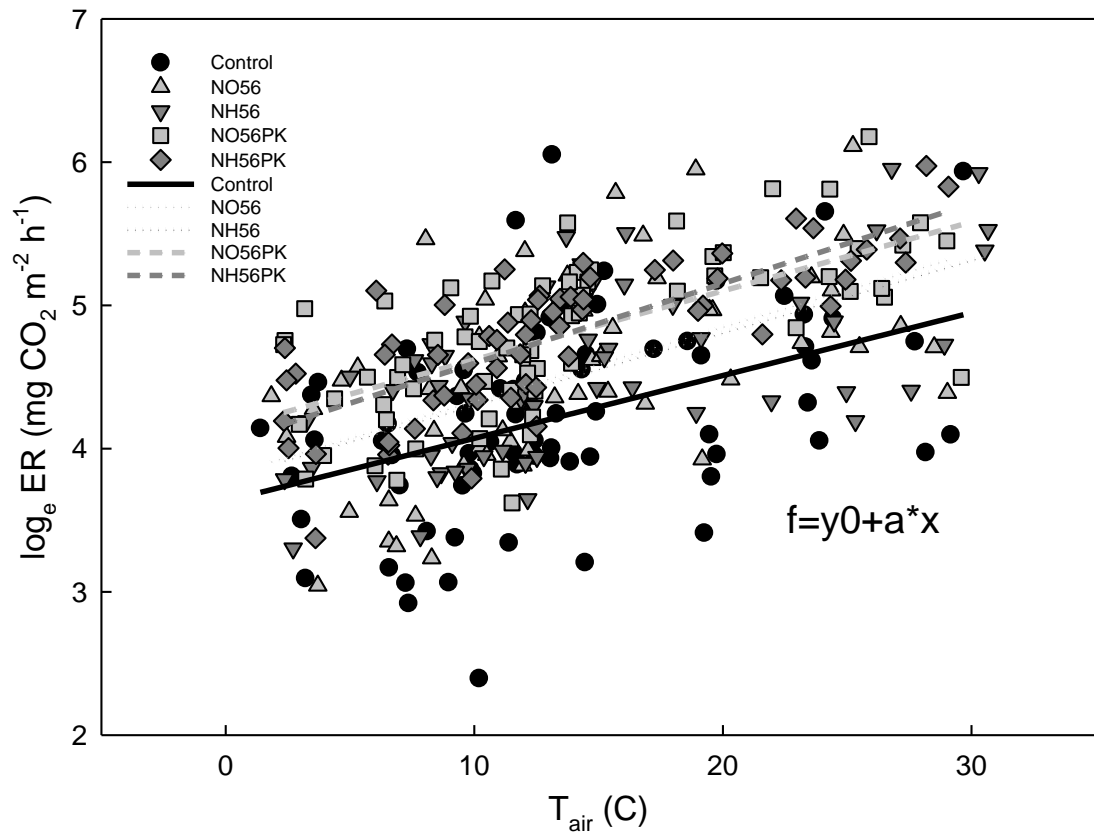


Figure 6.7. Log_e transformed ecosystem respiration (ER) values against air temperature. The figure shows that the respiration in the N addition plots is higher in a certain temperature than in the control plots and even more so in the NPK plots. This difference also is exacerbated with increasing temperature, i.e. the slope of the response for the treatments is steeper than for control.

Table 6.3. The intercept and slope values \pm SE when ER was plotted against air temperature and F and P values corrected for the mean of the observations.

Treatment	Intercept \pm SE	Slope \pm SE	F	P
Control	3.63 \pm 0.16	0.044 \pm 0.011	16.4	0.0001
NO56	3.81 \pm 0.17	0.052 \pm 0.011	23.10	<0.0001
NH56	3.80 \pm 0.13	0.051 \pm 0.008	37.13	<0.0001
NO56PK	4.14 \pm 0.11	0.049 \pm 0.007	47.34	<0.0001
NH56PK	4.04 \pm 0.087	0.056 \pm 0.006	96.63	<0.0001

Discussion

Overall C balance

At Whim bog the summer of 2008 was extremely wet and there were no serious drought periods, so the growth conditions for *Sphagnum* mosses should have been favourable. Regardless of this, all the plots were sources of CO₂, including the control plots. The results could be influenced by the assumption of 12-hour day year round. However, the results varied very little (less than 7%) when different day lengths were tested and therefore the estimation is believed to be reliable. This suggests that *S. capillifolium* stands at Whim bog are always a source of CO₂ and in fact, in a hot summer when temperatures rise higher they are probably even bigger sources of CO₂. Slightly lower ER (2.3-5.0 t C ha⁻¹ y⁻¹) values have been found in the UK in an afforested peaty soil (Zerva *et al.* 2005). Compared to values obtained in another study in the US by Carroll & Crill (1997) at a *Sphagnum* dominated poor fen the losses found in the present study are large (+4 to 5-fold). Also compared to other bogs in more natural condition, *Sphagnum* mosses at Whim are growing less and have smaller productivity (Chapter 4). The fact that the control plots are very large sources of CO₂, compared to other sites, raises concerns on the state of the bog in general. As it is, the annual rainfall is on the low side (Lindsay 1995; Ratcliffe 1964) and operations adjoining the site, rabbit grazing and tunnelling, together with human traffic round the plots may have deleteriously affected the continuity of the water supply to these hummock forming *Sphagnum*. These observations highlight the potential for N to exacerbate water stress. Also it is possible that centuries of human influence around the bog have been deleterious. Although the background deposition of 8 kg N ha⁻¹ y⁻¹ is small by British and central European values it is within the suggested range for critical load for bogs (5-10 kg ha⁻¹ y⁻¹) (UNECE 2003) and it might be that even the control plots are suffering from years of accumulated N deposition. In studies in Scandinavia, with lower background deposition, changes in the functioning of *Sphagnum* have been found for relatively low (10 kg N ha⁻¹ y⁻¹) increases in N deposition (Gunnarsson & Rydin 2000).

The present study is one of the few where the N deposition has been continued for several years. The results of short-term studies must be interpreted with caution and in fact, they show conflicting results (Saarnio *et al.* 2003; Gerdol *et al.* 2008). The only longer term study (more than 4 growing seasons) that we are aware of is a study by Bubier *et al.* (2007) in Canada where they studied a bog plant community of *Sphagnum* mosses, *Polytrichum* mosses and vascular plants. They found that in the high N treatments (60 kg ha⁻¹ y⁻¹) NEP was decreased unlike in this study where neither the 'N only' addition nor NPK addition had a significant effect on NEP. In the study by Bubier *et al.* (2007) the change in the NEP was due to a decrease in P_G which was not found in this study. In the present study in the 'N only' treatments, no effect of increased N on P_G was found but an increase in ER was found, although not statistically significant. In the NPK treatments both ER and P_G increased, although only P_G was statistically significant. Although no effects of 'N only' additions were found a larger accumulated loss of CO₂ during the year from all the treatments compared to control was found which is probably due to increased ER (Figs 6.6 and 6.7). The results from this study indicate that *Sphagnum* at Whim bog is P- or K-limited because adding NPK had an enhancing effect on ER and P_G whereas adding only N had no effect. Bubier *et al.* (2007) found very different results; they found that adding all three nutrients, NPK, decreased P_G with no difference in ER. However, their study included many different species unlike this study which focussed on *Sphagnum*.

Some studies have suggested that water table has a bigger influence on *Sphagnum* CO₂ fluxes than nutrients. In a study by Gerdol *et al.* (2008) where up to 30 kg of N ha⁻¹ y⁻¹ was added to an Italian bog it was found that during a very hot summer *Sphagnum* mosses were emitting CO₂ independent of the N treatment and it was not possible to disentangle the role of drought (temperature and water level) and nutrients. However, at the end of the 4-year experiment they found a weak stimulation of CO₂ efflux from the treatment plots suggesting that the experiment was too short to reveal the N effects. In the present study, we looked at the effect of

water table by plotting all the measured values against water table but no clear relationship was found. Also due to the very high rainfall, the differences in the water tables between plots and treatments were very small.

Changes in the fluxes between seasons

Both NEP and ER were larger in summer but the effects of season on P_G were less clear (Fig. 6.4). NEP was highest, i.e. least negative, during the spring and autumn and the only positive NEP values were measured in spring time. In spring and autumn time there could be an increase in P_G due to optimum moisture conditions for *Sphagnum* (Schipperges & Rydin 1998). There was also an indication of decreasing primary production of *Sphagnum* during high radiation levels, i.e. during summer, which could be seen in the calculated P_G first increasing with PAR but after a certain threshold starting to decline (Fig. 6.5). Murray *et al.* (1993) also found that high radiation levels lower the photosynthetic capacity of *Sphagnum*. High radiation levels are also often associated with high temperatures that themselves can be harmful (Breeuwer *et al.* 2009) and also might decrease the water content of the moss, which might in turn lower photosynthesis. The decrease in CO_2 assimilation is steep when the water content of *Sphagnum* decreases below the optimum for photosynthesis (Schipperges & Rydin 1998). However, very high water contents in *Sphagnum* also slow down the photosynthesis because the CO_2 diffusion path is increased (Schipperges & Rydin 1993); this might have affected the photosynthesis in winter time. Additional measurements at the site on *S. capillifolium* growing in full sun or shaded under *Calluna vulgaris* indicate complex interactions between N, waterholding capacity and photosystem II (Manninen *et al.* 2011).

During summer, and during high irradiance, very high ER values were measured which led to a substantial loss of CO_2 ; however, during the winter time ER values were lower leading to a smaller loss of CO_2 . Gerdol *et al.* (2008) found that *Sphagnum* mosses in the Italian Alps emitted CO_2 in a hot summer and also Saarnio *et al.* (2003) found decreased photosynthesis and increased respiration during hot and dry summer weather. It has also been found that wetting and hydrating cycles have a

negative effect on *Sphagnum* production (McNeil & Waddington 2003). Probably the most likely reason for the increase in ER during summer time was the increase in soil temperature and thus both the heterotrophic and autotrophic respiration. As expected, during winter when the temperature and radiation were low the measured fluxes were very small or non-existent as temperatures fell close to freezing.

Implications for climate change and C sequestration

This study clearly shows the detrimental effects of increased N deposition on *Sphagnum* and the C sink function of peat bogs. Even if the bog as a whole might be in a poor state the data still show that adding N makes the situation even worse and all the N treatments lost more CO₂ during the year than the control treatment. It seems that nitrate is worse for CO₂ loss than ammonium both with and without PK. The worse effect of nitrate is unexpected because ammonium has been found elsewhere to be taken up more effectively and be the more deleterious nitrogen form for *Sphagnum* than nitrate (Jauhiainen *et al.* 1998; Press & Lee 1986). As ER is a sum of both autotrophic and heterotrophic respiration, it is possible that the difference between the treatments is due to increase in the respiration of the microbial community or vascular plant roots. However, the difference between nitrate and ammonium could be seen both in ER and P_G; monthly average ER was higher during many months and the monthly average P_G is consistently lower in nitrate treatments, especially in the NO56PK (although this was not statistically significant). On the contrary, monthly average P_G in the NH56PK is in most cases the highest of all, which might explain the smaller CO₂ loss from this treatment. In the NO56PK treatments *Hypnum jutlandicum* has almost taken over the whole plot and *Sphagnum* microplots were difficult to find in these treatments. In NO56PK *Hypnum* is probably outcompeting *Sphagnum* but this has not happened in the NH56PK plots and might provide an explanation for the difference in the NPK treatments.

In this study an increasing temperature response of ecosystem respiration in the N treatments compared to control was found which was even more pronounced in the

NPK treatments. The consequences of this could be crucial for the C sequestration of bogs. With predicted increasing temperatures with climate change (Alcamo *et al.* 2007) it has been suggested that peatlands will in general turn into sources of C and therefore provide a positive feedback effect (Ellis *et al.* 2009; Moore 2002). Increased N deposition in many areas will make this problem worse although rising CO₂ might partly mitigate some of the increase in ER; *Sphagnum* growth and photosynthesis has been found in some studies to strongly increase with increasing CO₂ (Dorrepaal *et al.* 2004; Jauhiainen & Silvola 1999) However, Berendse *et al.* (2001) found no significant effect of increased CO₂ on *Sphagnum* biomass growth. It has also been predicted that with climate change in Britain the winter time precipitation would increase favouring *Sphagnum* and leading to possibly decreased ER and increased P_G but on the contrary the predicted warmer summers with less rain are going to increase ER and decrease P_G (Alcamo *et al.* 2007).

When evaluating the results from the present study it has to be taken into account that no measurements were done on rainy days due to risk of equipment malfunction. Clearly, this might bias the results. Regardless of this, this study clearly shows the effects of increased N deposition and the importance of long-term studies as the results obtained in this study are different to previous studies that have been short term. Some relatively short-term studies even suggest that *Sphagnum* can adapt to increased N deposition in the long-term (Limpens *et al.* 2003a) but this does not seem to be the case because even after six years of N fertilization there are very clear effects seen on this experiment.

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7. Other results

7.1. Water table

The water table in all the study plots fluctuated to a similar degree: in control plots between -25.4 cm and 8.2 cm, in the NO treatments between -18.4 cm and 9.8 cm, in the NH treatments between -22.5cm and 9.2 cm, in the NOPK treatments between -40.8 cm and 1.6 cm and in the NHPK treatments between -38.6 cm and -7.8 cm (Figs. 7.1 & 7.2). The slightly lower water tables in the PK treatments probably reflect different criteria used for placing the dip wells. Although the measured water table for the study plots was sometimes above the ground level, *Sphagnum* in the microplots was never submerged under water. In the PK plots the measurements started later, due to a lag in the dip well filling after inserting them. Because the difference in the water tables between the treatments was very small and most treatments showed within treatment variability, differences in water table were not expected to be important, especially since the relationship between the water table and water status of the hummock is not well quantified. Given both measurement years were very wet, with higher than average rainfall, between plot differences in water status would not be expected to be large enough to influence treatment comparisons.

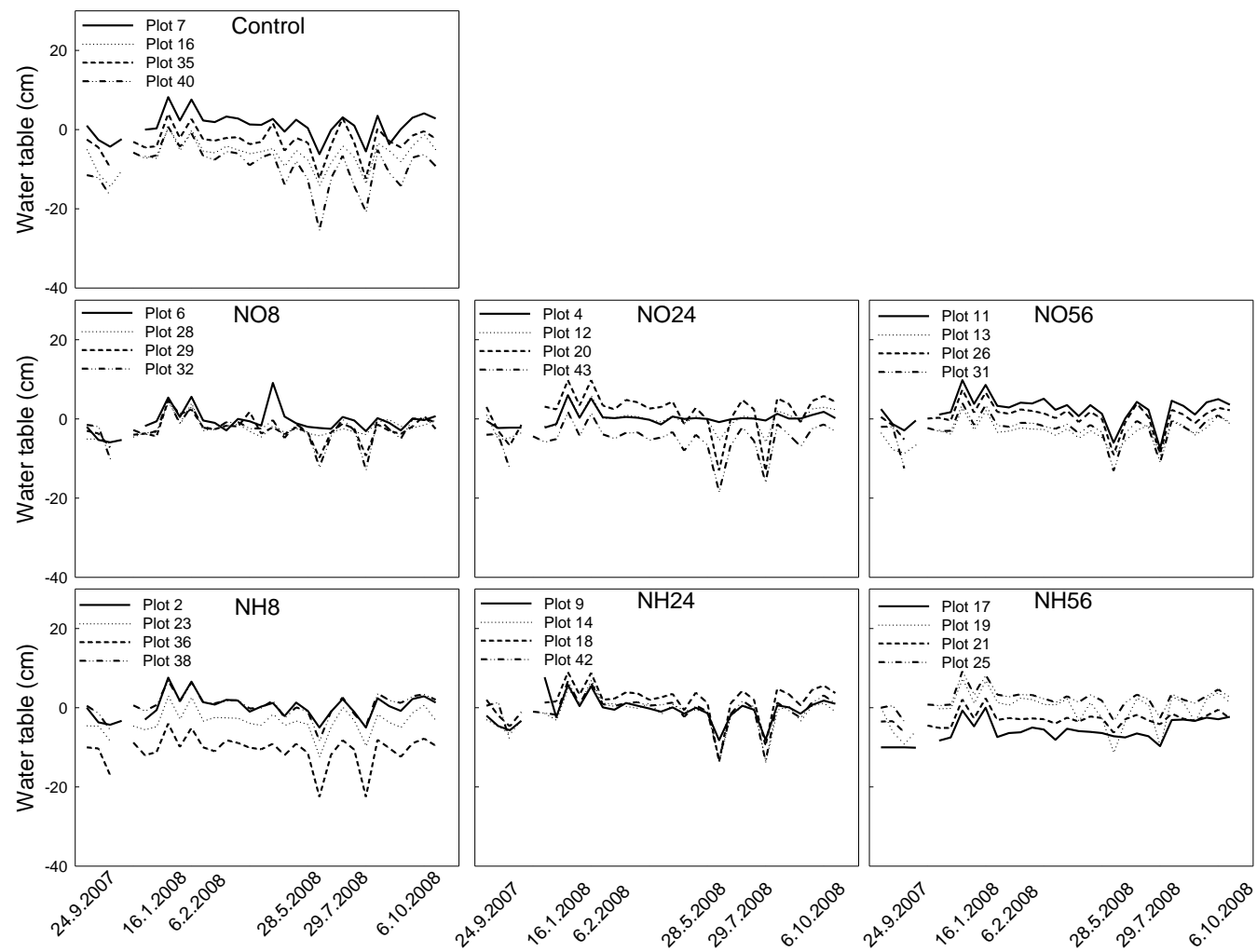


Figure 7.1. Water table at the control and “N only” plots between September 2007 and November 2008.

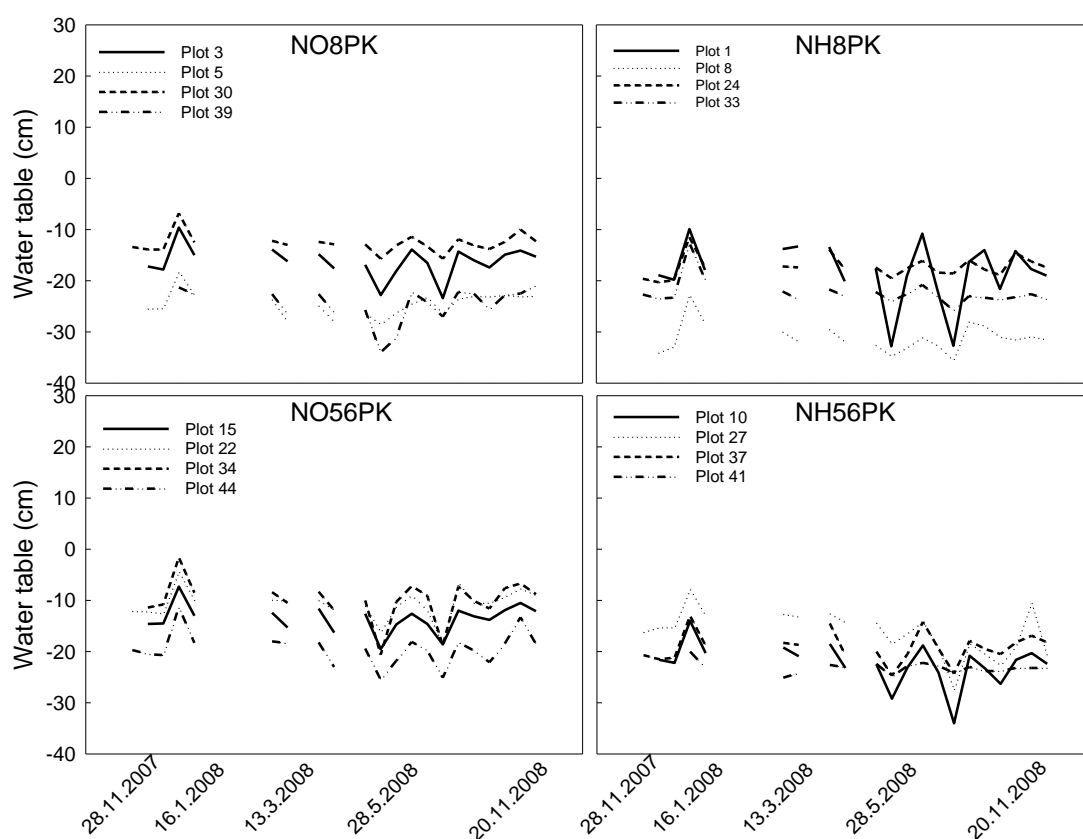


Figure 7.2. Water table at the NPK treatments between November 2007 and November 2008.

7.2. Vegetation description

Calluna vulgaris was the main shrub growing in the vicinity of the hummocks and *S. capillifolium* patches with the sedge *Eriophorum vaginatum* growing in the wetter flatter areas. Because the *Calluna* at the site is unmanaged its canopy tends to be sparse allowing *S. capillifolium* to occupy the understorey. Probably the main competitor to *S. capillifolium* at this site especially in the N treatments was *Hypnum jutlandicum* which tended to overgrow the *Sphagnum* especially in the high NOPK plots. The entire species list for every plot can be seen in Appendix IV. No difference in the amount of possible shading by vascular plants surrounding the microplots was found between the treatments (calculated as number of + in each plot). It is unlikely that shading either differed between the microplots or influenced the results as the

plots were selected deliberately, to minimise potential effects of shading, Thus, shading should not have caused differences in the results.

In the whole study plots (12.5 m²) between 2002 and 2009 *Sphagnum* (all species) cover increased in the control treatment by almost 30% (Fig.7.3) but decreased in response to N, particularly ammonium, with the biggest change in the NH56 plots (Fig. 7.3).

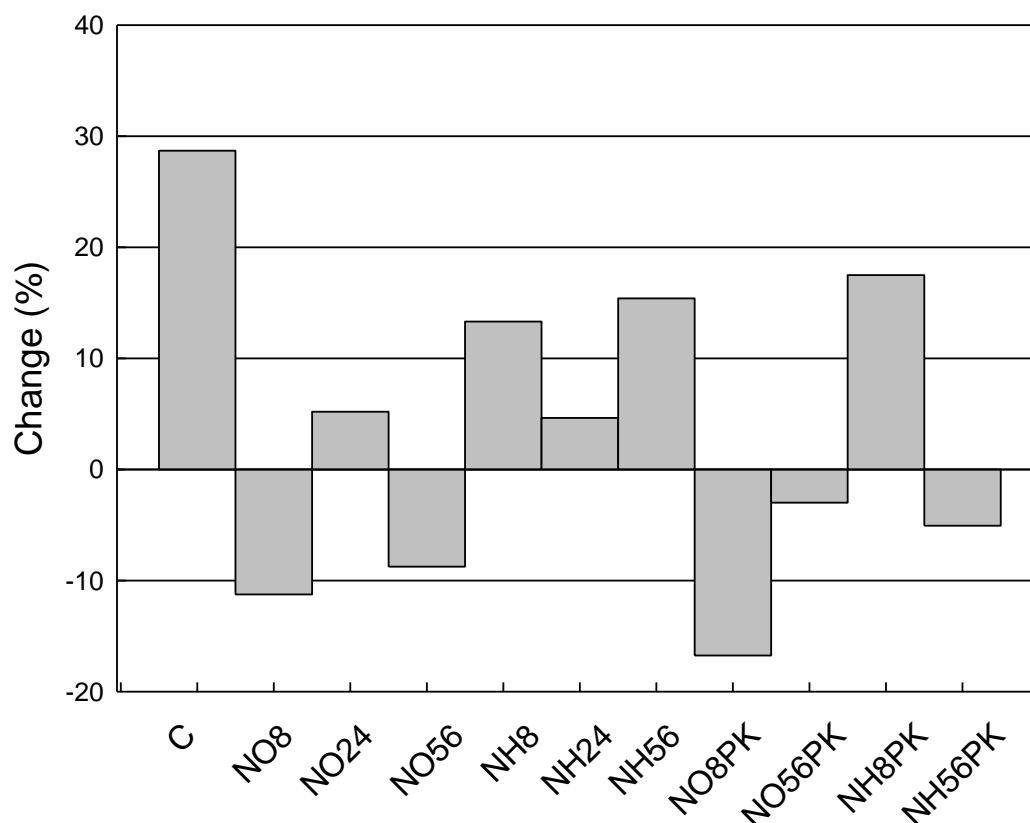


Figure 7.3. Mean percent change in the cover of *Sphagnum* growing in four 12.5 m² plots, between 2004 and 2009 in response to nitrate and ammonium treatments at 8, 24 and 56 kg N ha⁻¹ y⁻¹.

However, in the 2007 cover estimations for *S. capillifolium* from the permanent quadrats (3* 0.25 m² in each study plot) there was a clear response to N dose but not to N form (Figs 7.4 & 7.5). Also, with increasing ammonium dose the dose response became less obvious. With nitrate additions the cover in general changed very little with increasing dose and the effects changed at each assessment time (after 2, 5, and 7 years of treatment) (Sheppard L.J., pers. comm.).

Cover of *Calluna* was indifferent to both N dose and N form (Fig. 7.4 & 7.5). This supports the other results that there is no increased effect of shading for *Sphagnum* in the treatment plots compared to control plots. Also *E. vaginatum* was indifferent to N dose and form. For other mosses the changes have been inconsistent. *Pleurozium schreberi* cover was reduced by N dose, especially with ammonium, but *H. jutlandicum* appears to be tolerant of increased oxidised N and was even stimulated by the high dose (56 kg ha^{-1}).

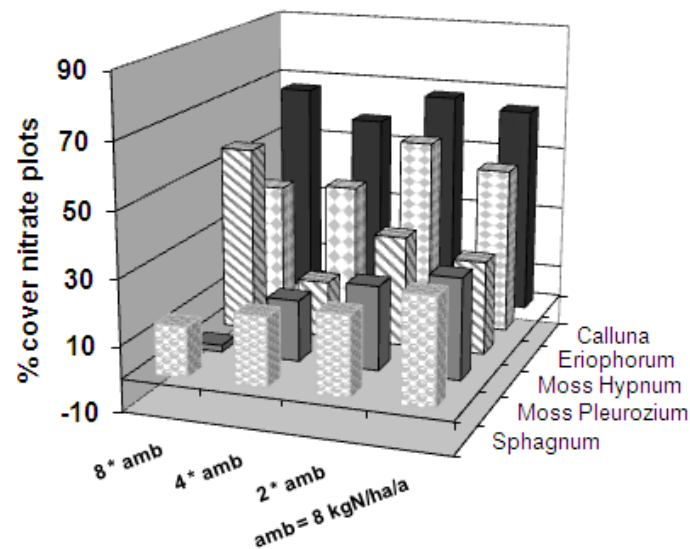


Figure 7.4. The cover of the most dominant species at Whim Moss in the control and nitrate plots after seven years of treatment.

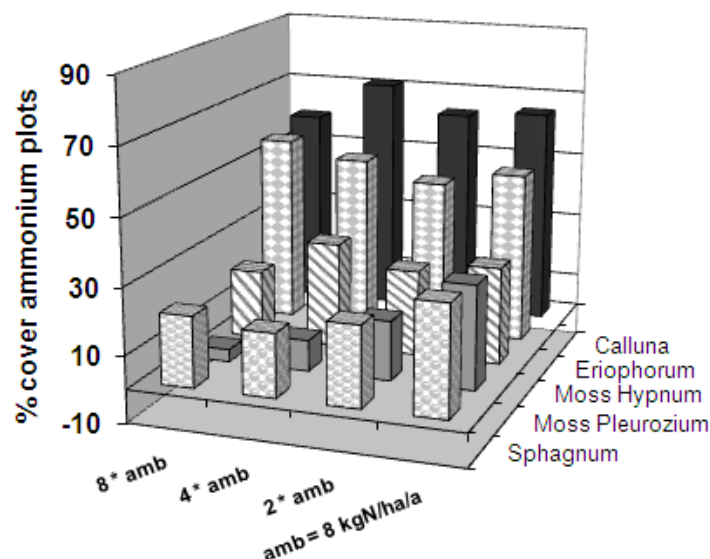


Figure 7.5. The cover of the most dominant species at Whim Moss in the control and ammonium plots after seven years of treatment.

Papers describing the changes in percentage cover of different species during the experiment are presented as appendices V and VI.

7.3. *Sphagnum* pH

Adding nitrate increased the pH of *Sphagnum* whereas the adding of ammonium decreased it (Fig. 7.6). However, with both forms the pH increases with increasing N dose. The trend was similar in the PK treatments; both form and dose affected pH. These measurements show that the increase (NO plots) and decrease (NH plots) in the pore water pH also affects *Sphagnum*. The change in tissue pH could possibly be one of the reasons explaining the changes in *Sphagnum* found in this study.

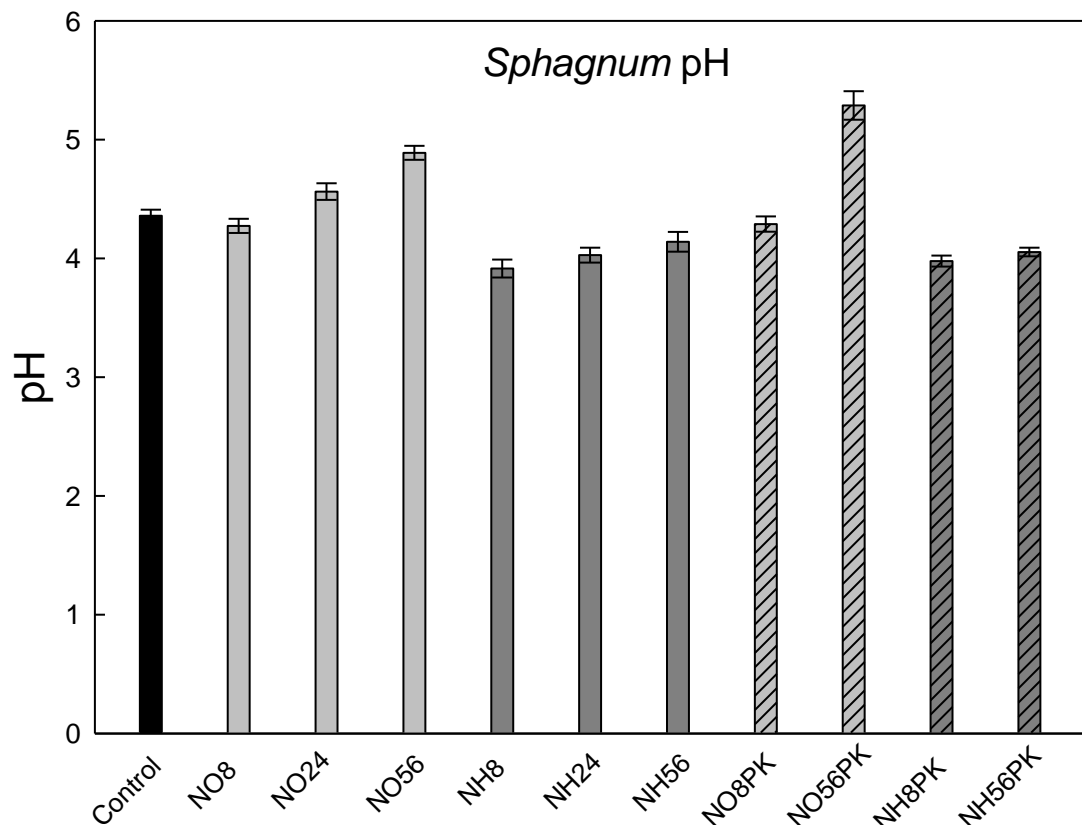


Figure 7.6. *Sphagnum* pH \pm SE measured on two occasions (the result is the average from all the plots for each treatment from both measurement times) from fresh *Sphagnum* samples mixed with deionised water and ground in a ball mill.

7.4. *Sphagnum* water content

The first few measurements (until 28.11.2007) had to be discarded from the PK plot data because of the lag in the dip wells filling up and uncertainties in the measured water table following from this. A linear relationship between water table and *Sphagnum* moisture, i.e. fresh weight/dry weight (fw/dw) ratio, was found in all plots except in plots 24 and 37 but the relationship in most of the plots was not very strong (Figs 7.7 & 7.8). However, the relationship between fw/dw and water table seemed to be steeper in the higher N treatments, i.e. in the high N treatments *Sphagnum* seemed to lose water faster than in the control plots indicating treatment with N reduced water retention. Adding nitrate seemed to reduce the retention capacity more than ammonium, i.e. the slopes were steeper in the nitrate treatments than in the

ammonium treatments (Table 7.1). Although the correlation between fw/dw and water table was relatively weak in some plots, this could explain why adding N decreases the growth. Indications of N disrupting the structure of the *Sphagnum* clumps were also found in the visual assessment (Paper I). Disruption of the structure and subsequent loss of water and moisture from *Sphagnum* can explain the lowering of shoot extension and productivity in the high N treatments. The loss of water holding capacity could also explain the worse effects of nitrate compared to ammonium on CO₂ fluxes.

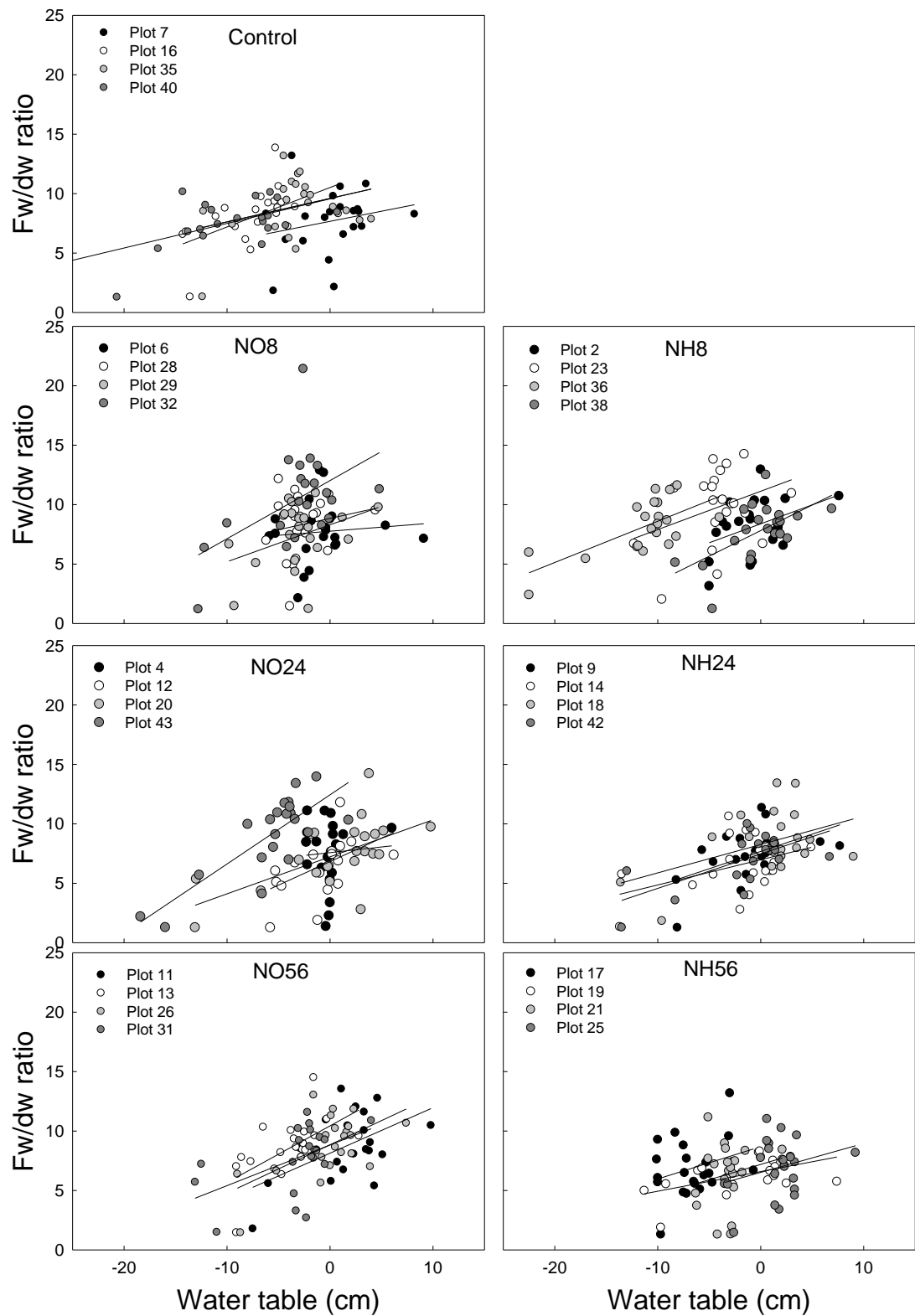


Figure 7.7. Measured *Sphagnum* fresh weight/dry weight ratio against water tables in the control and “N only” plots and the lines fitted for measurements from each treatment. The water tables were measured at the same time as the *Sphagnum* samples were collected.

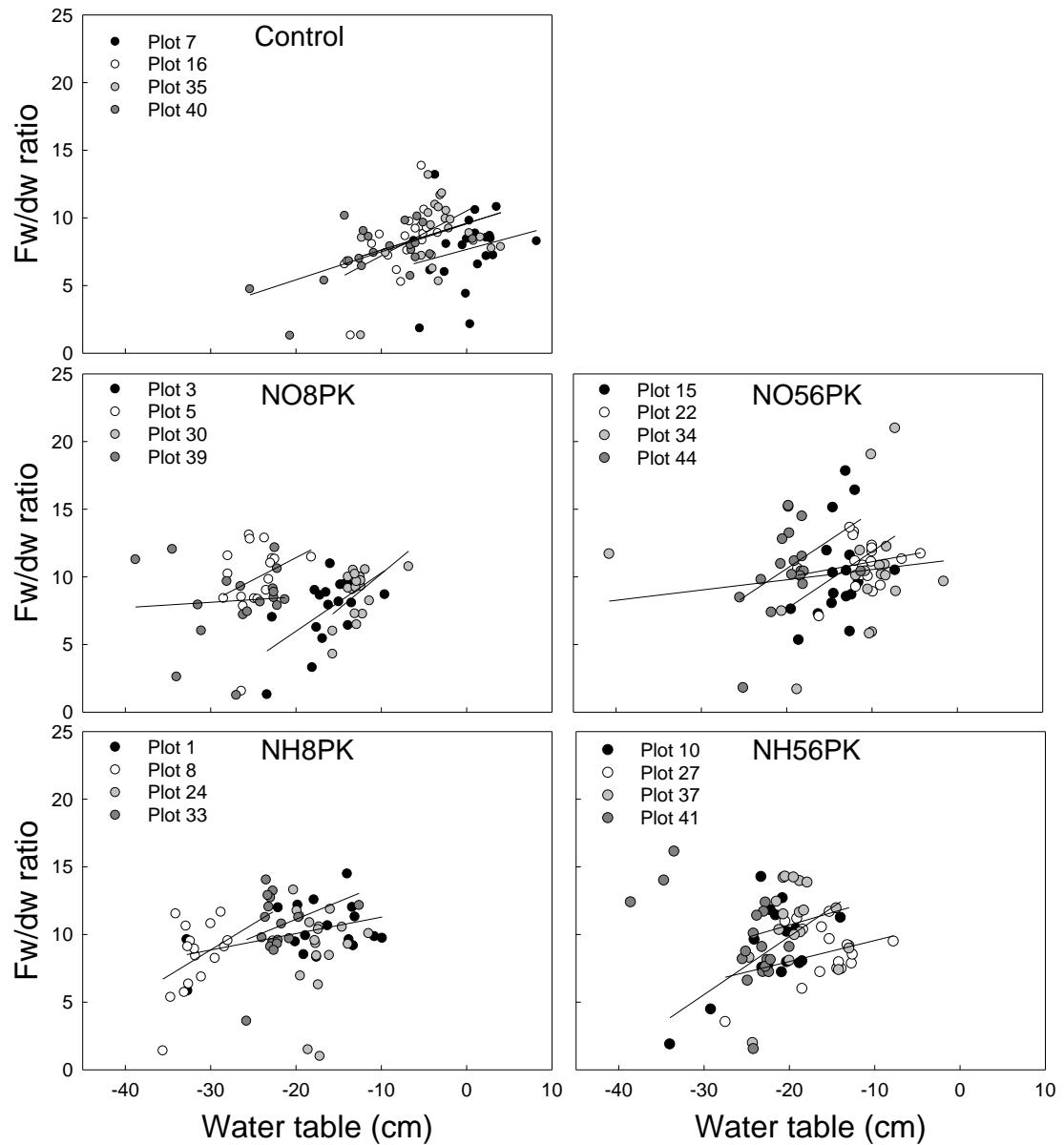


Figure 7.8. Measured *Sphagnum* fresh weight/dry weight ratio against water tables in the control and NPK plots and lines fitted to the measurements from each treatment. The water tables were measured at the same time as the *Sphagnum* samples were collected.

Table 7.1. The slopes, r^2 , Df and Pvalue of the fitted curves for water content against water table.

Treatment	Plot nr	Slope	r^2	DF	P-value
Control	7	0.1704	0.0478	2	0.3409
Control	16	0.3319	0.2785	2	0.0139
Control	35	0.1972	0.0993	2	0.1641
Control	40	0.2068	0.3585	2	0.0041
NO8	6	0.0687	0.0084	2	0.6926
NO8	28	0.191	0.0284	2	0.4652
NO8	29	0.3109	0.1459	2	0.0875
NO8	32	0.4893	0.2563	2	0.0192
NO24	4	0.0954	0.0037	2	0.7937
NO24	12	0.3953	0.2374	2	0.0251
NO24	20	0.3114	0.3896	2	0.0025
NO24	43	0.5819	0.6432	2	<0.001
NO56	11	0.3828	0.2747	2	0.0147
NO56	13	0.4767	0.3542	2	0.0044
NO56	26	0.4065	0.3406	2	0.0055
NO56	31	0.3419	0.2569	2	0.0190
NH8	2	0.3189	0.171	2	0.0624
NH8	23	0.3224	0.1068	2	0.1481
NH8	36	0.3473	0.4515	2	0.0009
NH8	38	0.4304	0.3609	2	0.0040
NH24	9	0.2908	0.2667	2	0.0165
NH24	14	0.2123	0.1813	2	0.0543
NH24	18	0.2386	0.2008	2	0.0416
NH24	42	0.2898	0.4224	2	0.0014
NH56	17	0.2717	0.083	2	0.2052
NH56	19	0.1654	0.283	2	0.0131
NH56	21	0.2579	0.0323	2	0.4353
NH56	25	0.2473	0.075	2	0.2295
NO8PK	3	0.4302	0.3389	2	0.0142
NO8PK	5	0.1912	0.0762	2	0.3006
NO8PK	30	0.5215	0.2925	2	0.0205
NO8PK	39	0.0396	0.0052	2	0.7786
NO56PK	15	0.423	0.1132	2	0.1867
NO56PK	22	0.1147	0.0455	2	0.3951
NO56PK	34	0.4426	0.1763	2	0.0933
NO56PK	44	0.4198	0.1752	2	0.0839
NH8PK	1	0.1213	0.1581	2	0.1140
NH8PK	8	0.3854	0.211	2	0.0636
NH8PK	24	-	-	-	-
NH8PK	33	1.052	0.6508	2	<0.001
NH56PK	10	0.429	0.3836	2	0.0080
NH56PK	27	0.155	0.1009	2	0.1989
NH56PK	37	-	-	-	-
NH56PK	41	0.1837	0.0323	2	0.4757

7.5. N₂O and CH₄

Both the CH₄ and N₂O fluxes were very small (Figs 7.9 & 7.10) and no difference between the treatments was found. In both measurement sets some values were positive and some negative, i.e. some plots were sources of N₂O and CH₄ (negative, plants loose) and some plots were sinks (positive, plants gain). Although the measurements were done only once, it seems that the contribution of these fluxes to the overall C and N balance of *Sphagnum* microplots was very small. The CH₄ flux is probably not coming from the *Sphagnum* plants but from below. It is also very likely that any CH₄ passing through *Sphagnum* would be oxidised to CO₂ which can explain the small flux. The CH₄ flux can be especially affected a lot by rain and the water level. However, as the water table was never above the moss surface in any of the treatments, the fluctuation in CH₄ is assumed to have been small. The measured N₂O flux was 10-fold to what has been otherwise measured from the site (Sheppard, L.J., pers. comm.) but even then, it is very small compared to the other N forms.

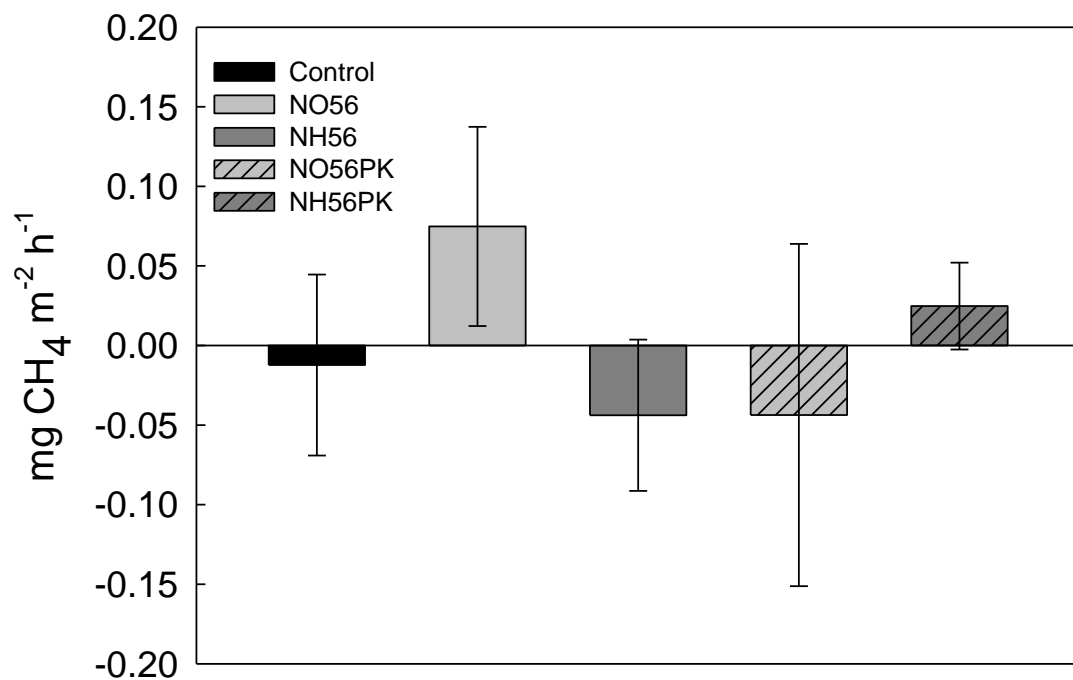


Figure 7.9. Measured methane fluxes in August 2008 \pm SE from the control and all 56 kg treatments.

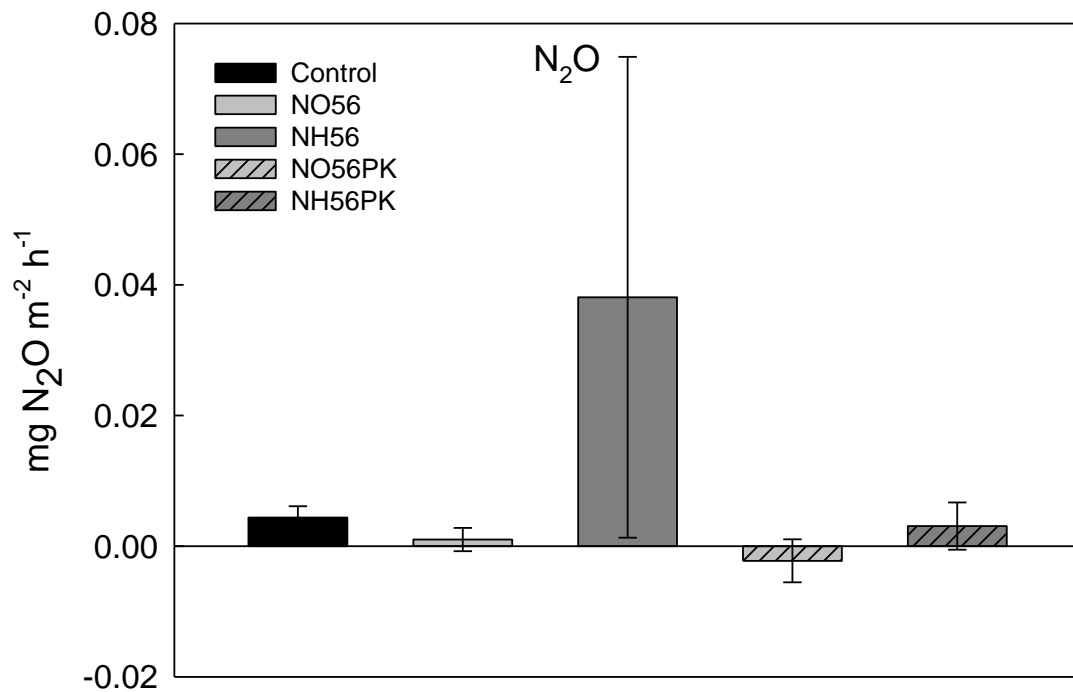


Figure 7.10. Measured N₂O fluxes in August 2008 ± SE from the control and all 56 kg treatments.

7.6. Diurnal CO₂ measurements

The diurnal measurements were undertaken on an atypical very hot day and the *Sphagnum* was clearly stressed; all the calculated P_G values were negative (except one value for control plots) (Fig. 7.11). A negative P_G value is possible because it is not a measured value but is calculated from the difference between the measured NEP and ER values. In practice this means that temporarily ER exceeds NEP, which obviously is not sustainable for long periods of time, as *Sphagnum* could not grow. The largest ER values were measured during the night and at midday when the radiation was at its highest. Respiration in the control plots is smaller and P_G higher than in the N treatment plots throughout the 24-hour measurement period. P_G remained at the same level throughout the period of light; the time of day when the other field measurements were done should therefore not affect the results. This suggests that *Sphagnum* P_G is relatively insensitive to the quality of light and might partly explain the lack of response to PAR in all the measurements (Fig. 6.5).

However, for ER a large difference between day and night was found and the ER measurements done during the day probably underestimate the amount of ER during night time. If this is the case, the calculated CO₂ balances are underestimating the amount of CO₂ released from these plots.

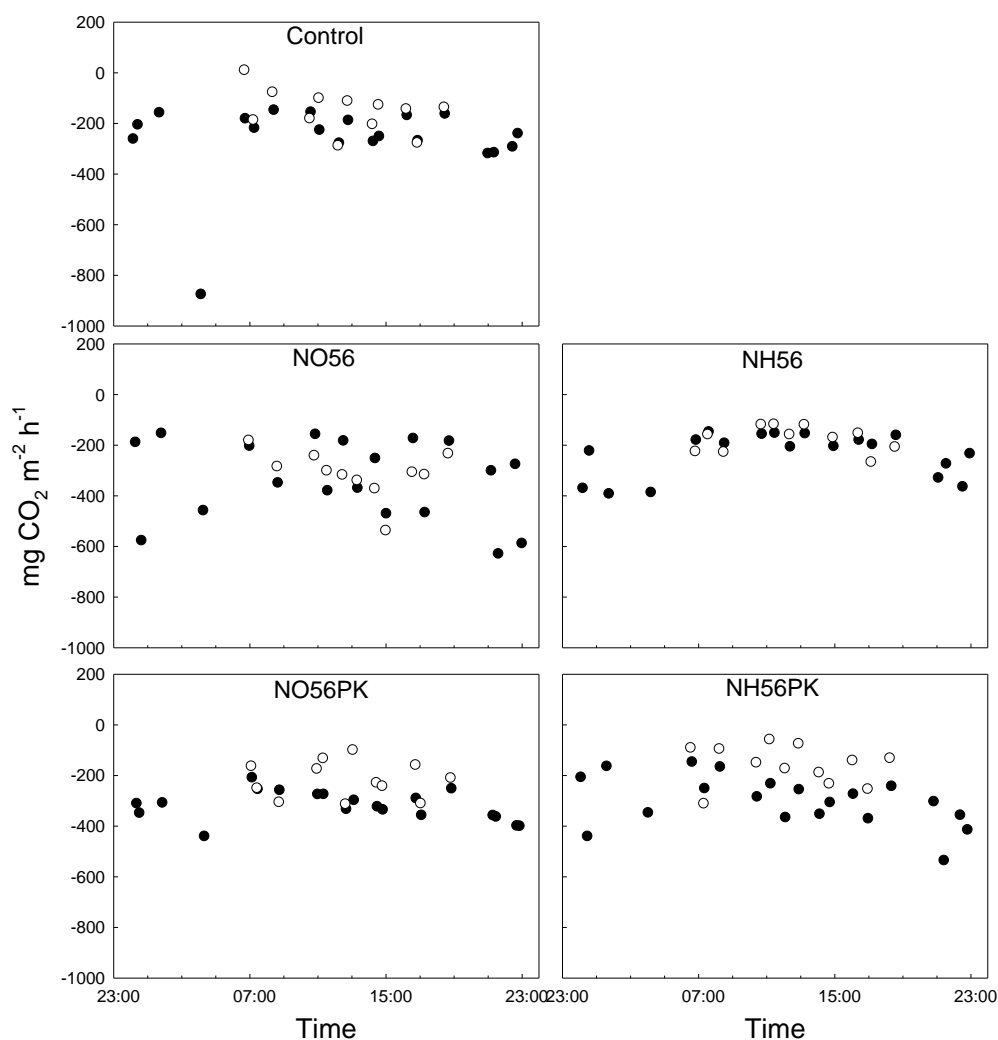


Figure 7.11. Measured P_G and ER fluxes during the 24-hour measurement period in July 2008. Open circles represent P_G measurements and dark circles ER measurements.

7.7. C and N balance

All the treatments were sources of CO₂ during year 2008. They also released C as DOC and although some of them were sinks of CH₄, the CH₄ fluxes were extremely

small and had no effect on all the treatments being sources of C during the year. The main contributor was CO₂ but also DOC had an important role (Table 7.2). The biggest release of C happened during summer due to higher release of DOC (Paper II) and CO₂-C (Paper III) (Fig. 7.12). Many assumptions have been made in calculating the rate of loss of DOC from the measured concentrations and CH₄ flux was only measured once. However, these values are not meant to represent the long-term means but to give an indication of the relative sizes of different fluxes.

Table 7.2. The amount of C released as CO₂-C, CH₄-C and DOC and bind in productivity to new plant mass from the treatments during year 2008.

Treatment	CO ₂ -C (g m ⁻² y ⁻¹)	CH ₄ -C (g m ⁻² y ⁻¹)	DOC (g m ⁻² y ⁻¹)	C bound in <i>Sphagnum</i> (g m ⁻² y ⁻¹)
C	-177	-0.00011	-26	106
NO56	-220	0.00067	-17	32
NH56	-197	-0.00040	-25	36
NO56PK	-265	-0.00040	-43	38
NH56PK	-215	0.00022	-19	87

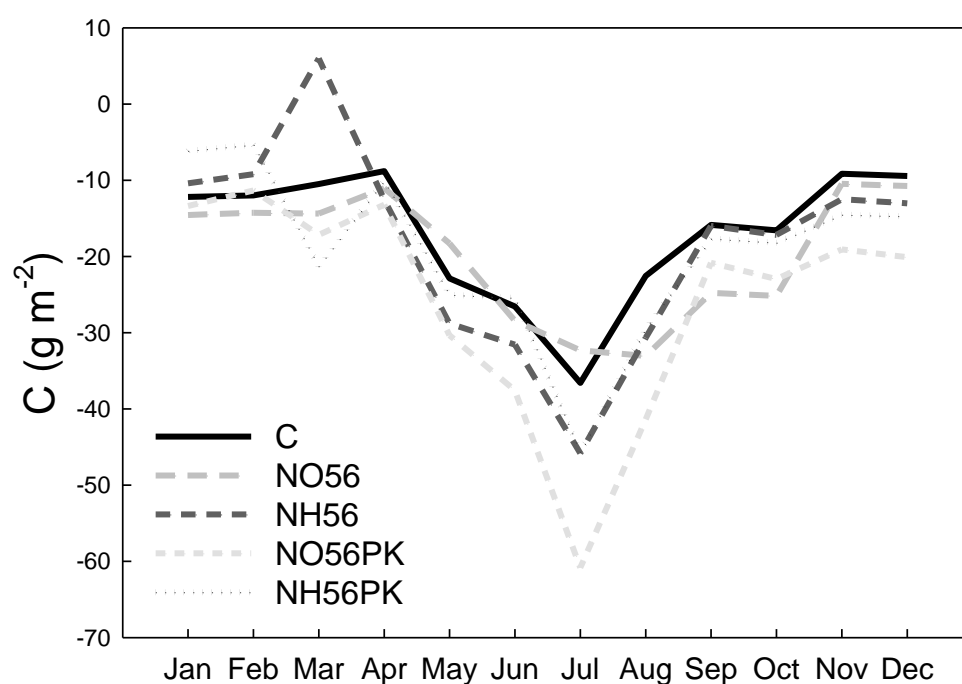


Figure 7.12. The pattern of C release from the different treatments during 2008. This is calculated as a sum of monthly average CO₂, DOC and CH₄ fluxes. CH₄ flux was only measured once and the same amount is used for all months.

N₂O fluxes were extremely small and most N that was deposited was released in pore water or bound to new plant tissue (Table 7.3). In the control treatment the amount of N bound to plant tissues and released in pore water is greater than the N deposition. In the other treatments the amount of N released in pore water and bind in new plant material was actually less than the deposited amount. As with C, many assumptions had to be made when calculating the flux of N in pore water from the measured concentrations and N₂O flux was only measured once. However, both the C and N balance should provide a scale and make it possible to compare the importance of different fluxes.

Table 7.3. The amount of N deposited (background deposition + treatment) , released as N₂O-N and N in pore water and bind in productivity to new plant mass from the treatments during year 2008.

Treatment	N deposition (g m ⁻² y ⁻¹)	N ₂ O-N (g m ⁻² y ⁻¹)	N in pore water (g m ⁻² y ⁻¹)	N in plants (g m ⁻² y ⁻¹)
C	0.8	0.017	-0.66	2.00
NO56	6.4	0.0040	-4.24	1.01
NH56	6.4	0.15	-1.96	1.42
NO56PK	6.4	-0.009	-1.52	0.99
NH56PK	6.4	0.008	-0.65	2.70

8. General discussion

The aim of this study was to compare the effects of wet oxidised and reduced N, at doses that are representative of N deposition in the UK, on one of the keystone species growing in ombrotrophic bogs. Peat forming *Sphagnum* species are particularly valued for the role they play in C sequestration and the end product, peat, is the foundation of many ecosystems of high conservation value, especially in Scotland (Lindsay 2010). In addition, the study sought to identify indicators of N effects that could be used by the Conservation Agencies to determine whether peatland ecosystems were being detrimentally impacted by N deposition. Reactive N deposition is widely recognized as a major cause of biodiversity decline among semi-natural ecosystems but are the N deposition loadings that Scottish peat bogs are subjected to going to adversely affect *Sphagnum* and the sustainability of peat bog ecosystems?

This study has addressed this question using just one hummock forming species and recognizes the results may not apply to pool and lawn *Sphagnum* species. Hummocks appear to trap more inorganic nutrient particles than hollows (Oldfield *et al.* 1979; Jauhianen *et al.* 1998) which makes them more susceptible to increased N deposition. In addition, hummocks of *S. capillifolium* appear to grow better than other hummock forming *Sphagnum* species due to their superior water-conducting ability (Clymo & Reddaway 1972). It has also been found that the subspecies *S. rubellum* is less sensitive to N than for example another hummock species *S. fuscum* (Clymo & Reddaway 1972) These observations suggest that *S. capillifolium* (including both subspecies *capillifolium* and *rubellum*) may be one of the more N tolerant hummock forming *Sphagnum* species so that N thresholds based on the performance of this species in the field, provide an upper estimate for tolerance which would probably need to be lowered for other hummock species, when adopting the precautionary approach to ecosystem protection.

Most of the work was conducted at Whim bog where CEH have been manipulating the form (oxidised versus reduced) of wet N deposition to National Vegetation

Classification M19 vegetation (*Calluna vulgaris*- *Eriophorum vaginatum* blanket mire) (Rodwell 1991). This study has looked at the C balance through growth (cranked wires), NEP measurements (small chambers on permanently installed bases, 10 cm deep cylinders), decomposition (litter bags), DOC in leachate (rhizon samplers), vitality (visual) and nutrient contents (C, N, P, K, Ca, Mg) (in years 5 and 6 of treatment). Other studies at the site have examined change in cover of *Sphagnum* and all other species, growth, phosphatase activity and a range of changes in soil chemistry. In this overview these studies will be referenced when appropriate for the interpretation of the findings from this PhD study.

8.1. Reliability of the methodologies

The manipulation experiment that provides the backbone of this study is globally unique with respect to the treatment scenarios adopted and the detail and procedures have been peer reviewed (Sheppard *et al.* 2004). Treatment plots were large and, for a field experiment, well replicated. However, the vegetation at Whim bog is highly spatially heterogeneous so that replicate plots were not identical and while every attempt was made to identify similar hummocks in each plot, this was not always possible. This meant that for effects to be significant, they needed to be quite large. The experimental treatments had been applied for five to six years prior to the measurements reported here, enabling the study to evaluate more longer – term changes, and the potential for acclimation.

Growth, shoot extension and productivity measurements were made using the cranked wire method (Clymo 1970), modifications of which have been very widely used. The number of wires used in this study was relatively high compared to many other studies (Appendix II), to improve the precision and reduce the influence of spatial heterogeneity. Litter bags, again another widely employed technique were used to look at decomposition rates. However, there are many ways that the technique can vary, which can undermine interstudy comparisons: where the litter is taken from, where the bag is placed and the size of mesh determines which organisms are excluded. The methodology used here, took account of these issues,

with in-growth and loss of material being the only problems observed. These were minimized by carefully removing ‘foreign’ material from the retrieved bags before drying and weighing, although even after this a mass increase was measured for some bags. The CO₂ measurements were undertaken throughout one year and in all feasible weather conditions (rain and extreme cold affect the power supply so no measurements could be made when wet or frozen) and at different times of day. Although longer measurement periods would be ideal for all these measurements, this was not achievable within the timeframe of the project.

8.2. Does N pose a threat to carbon sequestration?

It was hypothesised that excess N would threaten the sustainability of peatlands through detrimental effects on *Sphagnum*. Only one species of this large genus grew in sufficient abundance at the experimental site, but *S. capillifolium* is a widely distributed hummock forming species in the UK and an important component of M19 ombrotrophic bogs (Averis *et al.* 2004; Lindsay 2010).

Shoot extension was reduced in response to N additions irrespective of N dose, *i.e.* all N doses reduced extension growth by a similar amount. The effect of form was not significant although, 8 kg ha⁻¹ y⁻¹ N supplied in the oxidised form was less detrimental than the reduced N form (Chapter 4). These results, for ammonium at least, concur with the current critical N load for bogs of 5-10 kg N ha⁻¹ y⁻¹. Increasing N dose also increased %N in the plant tissue. The threshold for negative changes for *Sphagnum* productivity has been suggested to be up to 20 mg g⁻¹ (Lamers *et al.*, 2000; Berendse *et al.*, 2001; Limpens *et al.*, 2003a). However, in this study the N concentration stayed below 2% in all the treatments despite reducing productivity. The results from this study suggest that the earlier estimations for the threshold for negative effects are too high and that with longer exposure times the thresholds are lower. The lowest N dose applied (~8 + 8 kg N ha⁻¹ y⁻¹) decreased the productivity of *S. capillifolium* to 243 g m⁻² y⁻¹ in the control compared with 164 g m⁻² y⁻¹ in NO8 and 157 g m⁻² y⁻¹ in NH8. This concurs with the critical load of 5-10 kg N ha⁻¹ y⁻¹ for ombrotrophic bogs (Bobbink & Roelofs, 1995; Bragazza *et al.*,

2004) as already with the dose of $16 \text{ kg ha}^{-1} \text{ y}^{-1}$ a large reduction in *Sphagnum* productivity was found. The threshold for changing the effects from positive to negative for *Sphagnum* productivity, if a threshold exists, is therefore $<16 \text{ kg ha}^{-1} \text{ y}^{-1}$. This is below the estimate of $18 \text{ kg ha}^{-1} \text{ y}^{-1}$ that some previous studies have suggested (Lamers *et al.*, 2000; Berendse *et al.*, 2001; Limpens *et al.*, 2003a) but in line with Gunnarsson & Rydin (2000) who suggested a critical load $<10 \text{ kg ha}^{-1} \text{ y}^{-1}$.

However, the different criteria used to evaluate the N response in terms of growth led to different N dose thresholds and different effects of N form. This may in part reflect interactions with climate and interannual differences in rainfall particularly (Carfrae *et al.* 2007), with respect to when cover assessments were made. It should be pointed out also, that it is notoriously difficult to establish whether *Sphagnum* is irreversibly damaged and whether it will recover. The score given in the visual assessment of damage decreased with increasing N dose, indicative of more damage with increasing N dose and cover estimates also imply a dose effect, although plot cover was least affected by reduced N at $8 \text{ kg N ha}^{-1} \text{ y}^{-1}$ (Sheppard *et al.* 2010).

A more accurate assessment of changes in cover, using three 0.25 m^2 permanent quadrats and pretreatment cover as a covariate, also show how the responses do not remain consistent over time and are highly variable. The permanent quadrat data suggests that with increasing accumulated reduced N dose the dose response of *Sphagnum* (all *Sphagnum* species present used) becomes less obvious. After 7 years in the $8 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ammonium treatment *Sphagnum* cover had started to decline and in the NH24 treatment had declined to the similar reduced cover observed with NH56 (Sheppard L.J., pers. comm.). By contrast treatment with oxidised N showed no clear N dose signal but *Sphagnum* cover varied depending on whether it was assessed at 2, 5 or 7 years after treatment started and overall cover changed by very little as a consequence of oxidised N inputs (Sheppard L.J., pers. comm.). In the early years of the experiment, after two years of treatments, Carfrae *et al.* (2007) looked at different measures of *S. capillifolium* performance, and reported a significant reduction in side branch production in response to the high doses of both oxidised and reduced N (lower doses not assessed), a decrease in the length of the

green photosynthetic shoot with nitrate but no significant effect on shoot dry weight. In this study adding N decreased shoot extension and productivity, but there was no statistical dose effect, probably reflecting differences in growing conditions between the different plots giving rise to large error bars.

The results for cover in plots receiving oxidised N have shown no consistency in response to N dose. However, the NEP data presented in Chapter 6 showed that nitrate increased the ecosystem respiration more than ammonium, which in time could lead to reductions in cover. This is surprising because ammonium was expected to be more detrimental for *Sphagnum* because it is more readily available and taken up in higher quantities (Jauhianen *et al.* 1998; Wiedermann *et al.* 2009). The smaller CO₂ flux from ammonium plots might be due to the decrease in pH compared to an increase with nitrate. An earlier study by Aerts and De Caluwe (1999) concluded that N deposition leads to soil acidification which leads to reduced CO₂ emission. This suggests that for the CO₂ balance the form of N deposited is extremely important. Interestingly Carfrae *et al.* (2007) also showed that high nitrate can significantly reduce the amount of photosynthetic material, possibly as a consequence of increased photo-oxidative stress under desiccating conditions (Manninen *et al.* 2011). By contrast through the greater N accumulation measured with ammonium the likelihood of toxicity is increased. Data for amino acids (van den Berg, unpubl.), which are significantly higher in the reduced N treatments further support the risk of toxicity with ammonium.

Despite the absence of consistent statistical effects this study has provided valuable complimentary data to what has been collected since the manipulation began in 2002, greatly increasing our understanding of the importance of N dose and form and how N affects *S. capillifolium*. This study shows that after 5 years of increased N any stimulatory effects of N at the low dose were only transitory and have been offset through deleterious effects of the accumulating N dose. If as suggested by the cover data *Sphagnum* is responding to the cumulative dose, then maintaining the treatments and assessments at Whim should be a priority. Equally, disagreement over the response thresholds emerging from the various studies may relate to the level of

background deposition where the study is undertaken and also which N form dominates the deposition.

In terms of growth, vitality and cover, and carbon acquisition and loss, these results confirm that N doses above the critical load will have detrimental effects on *Sphagnum*, based on the 8 year experiment at Whim. However, with respect to carbon sequestration and losses and the maintenance of our peatlands, the role of N deposition in decomposition, mineralization, gaseous and fluvial losses of C as CO₂ and DOC must also be considered.

As was discussed in Chapter 5 the studies showing that N increases decomposition in bogs are not wholly convincing as there is a scarcity of field data. Contrary to some studies that have shown increased decomposition rates with increased N *e.g.* Bragazza *et al.* (2006), this study has shown that decomposition was slowed in the presence of elevated N. Bragazza and Freeman (2007) recorded lower phenol concentrations in N treated *Sphagnum*, and attributed their faster decomposition rates to this. Recent measurements of phenol concentrations in *Sphagnum* at Whim (Ochoa R., pers comm.) show N has increased phenolic concentrations which is consistent with the slower decomposition rates based on Bragazza and Freeman's hypothesis 2007. However, Hajek *et al.* (2010) have contested the role of phenolics in controlling decomposition rates, on the basis of results from a stepwise analysis of the effects of the different biochemical constituents of *Sphagnum* litter on decomposition rates. Lindsay (2010) in a recent review of the current state of UK peatlands reports that for many bogs, it is their low N content that is responsible for the slow rates of *Sphagnum* decomposition. Given the importance of decomposition rates for C sequestration *i.e.* peat formation in ombrotrophic bogs, the role of N and its effects on the biochemistry of *Sphagnum* litter warrants more research to establish how elevated N deposition influences decomposition rates in bogs.

Peatlands provide an important ecosystem service by storing C; the amount of C stored in peatlands is in the same magnitude as C fixed as oil or natural gas (Keddy *et al.* 2009). Changes in the C balance of peatlands can lead to changes in the global

C budget. Therefore, when evaluating the effects of N deposition on bogs it is extremely important to make estimates of the effects on the complete C balance. In the present study at Whim bog the C balance was dominated by the CO₂ flux which exceeded the C lost as DOC, approximately 8-fold. The C lost as methane from these hummocks, which at Whim are often much higher than the water table, was extremely small. There are several possible explanations for this: 1) vascular plants, especially sedges, are large emitters of methane (Thomas *et al.* 1996) and these were excluded or 2) in a recent study Chen and Murrell (2010) report the presence of aerobic methane oxidising bacteria which live in symbiosis with *Sphagnum* and convert CH₄ to CO₂, which is then taken up by *Sphagnum*. Although hummock forming *Sphagnum* form the least effective symbioses they may contribute to the low methane emissions measured. In a study on fen peats CH₄ emissions have been found to increase with N deposition, as nitrate can be used as an electron acceptor (Aerts & De Caluwe 1999). Indeed at this site static chambers inserted into the peat, avoiding the hummocks, have shown enhanced methane emissions with nitrate (Sheppard *et al.* 2008). DOC losses were not significantly affected by N form or dose, although there was a tendency for DOC concentrations to be lower in pore water from the N treatment plots. There is no evidence therefore that N increases gaseous and fluvial losses except for CO₂ from *Sphagnum*.

8.3. Effects of PK when added with N

The majority of semi-natural ecosystems have evolved and adapted to low N availability. In many cases the availability of other major nutrients *i.e.* P and K is also low, contributing to constraints on growth. Given that these other nutrients are not increased in balance with N through the process of N deposition it was hypothesised that increasing the supply of these nutrients commensurate with N might mitigate some of the more deleterious N effects, and could even provide a management tool to counteract N deposition effects under particular circumstances.

The vitality score for *Sphagnum* in the visual health assessment improved when PK was added with N. The improved condition of *Sphagnum* with PK additions was also

reflected in enhanced shoot extension and productivity, where the negative effects of N were alleviated with simultaneous PK additions. Adding PK with N decreased %N in the plant tissue, suggesting that at Whim growth was co-limited by N and P+K (effects not separable). Enabling growth, which diluted the N uptake and restricted the likelihood of N accumulating, especially ammonium in toxic amounts, probably explains the improved shoot extension and productivity when N was added with PK.

However, *Sphagnum* was not the only species to benefit from the PK additions. Hypnaceous mosses, especially in plots receiving nitrate, also grew much better and often overgrew *Sphagnum*. Thus although PK additions reduced the direct negative effects of N, PK exacerbated detrimental indirect effects, *i.e.* competition. Also the CO₂ flux measurements registered increased respiration which could have been an effect on *Sphagnum*, but may also represent higher respiration by roots and soil organisms. Decomposition rates, after 18 months of incubation, were also slightly less restricted by N additions when PK was supplied. Bubier *et al.* (2007) suggested from their study that PK might even be more toxic than N and the concept of P toxicity has recently been evaluated by Lambers *et al.* (in press). Certainly the available evidence does not support the view that improving PK supply to N impacted bogs will improve conditions for *Sphagnum* despite enhancing the capacity of *Sphagnum* to assimilate N (Limpens *et al.* 2004).

8.4. Role of climate

The significance of climate as a modifier of the effects of N deposition was not explicitly tested in this study. However, while field studies such as this one, cannot control climate they can capitalise on weather events, through measurements of potential driving variables: rainfall, temperature etc. Two exceptionally dry periods, particularly the summer of 2003, and to a lesser extent 2006 occurred during the experiment which have shown that desiccation can exacerbate detrimental effects of N, though rarely permanently (Carfrae *et al.* 2007). The annual rainfall at Whim was below 1200 mm in these years so it is possible that the *Sphagnum* at Whim is growing at the edge of its tolerance range (Ratcliffe 1964; Lindsay 2010) making it

particularly sensitive to detrimental effects associated with elevated N. Measurements made in this study however, were undertaken during unusually wet years. Therefore, the results for shoot extension and productivity can be assumed to represent normal or even favourable years and in drier years C fixation would be reduced, *i.e.* *Sphagnum* mosses at the study site would probably grow less and thus bind less C.

8.5. Implications for the sustainability of Scottish peatlands?

In addition to their huge conservation value peat based bogs can represent thousands of years of organic matter accumulation and are amongst Britain's most ancient natural/semi-natural ecosystems being valued for their specialised plant and bird communities. In the last 40 years there has been a change in the public opinion about wetlands and their importance (Keddy *et al.* 2009) and peatlands are now particularly valued for the ecosystem services they provide (Millennium Ecosystem Assessment 2005). Some ecosystem services increase by area, for example C sequestration which is a function of peat area times peat depth, so the size of these ecosystems matters as well (Keddy *et al.* 2009; Roulet 2000). Distance of road net work correlates negatively with biotic diversity in wetlands (Houlahan *et al.* 2006) and also decreases the N deposition. The number of species also increases in relation to the area of the wetland (Findlay & Houlahan 1997). These things together make it very important to conserve large peatland areas as found in Scotland and to avoid further fragmentation.

In Scotland bog and peatland ecosystems are found predominantly in the wetter north and west and thus receive most of their N load as wet deposition. In Europe, many bogs occurred in regions of intensive livestock farming; here the detrimental effects of reduced nitrogen, ammonia and ammonium, have transformed such bogs into grassy plains (Heil and Diemont 1983). The majority of British bogs, with the exception of those in Northern Ireland, by comparison do not occur in areas of high nitrogen deposition, although most sites already receive the Critical Nitrogen Load of 5-10 kg N ha⁻¹ y⁻¹. Scottish bogs which occur in areas characterised by high rainfall

will see N deposition loads approximating to the Critical Load, but at low concentrations by virtue of the large amount of rain. The Whim experiment used relatively low ionic concentrations of nitrate and ammonium and these were generally applied to wet vegetation as result of the coupling between treatment and rainfall to minimise the influence (artefact) of ionic strength on the measured effects (Pearce and van der Wal 2008). The background N deposition at Whim is similar to or greater than that experienced by the majority of Scottish ombrotrophic bogs (Fig. 1.1) and the relatively low rainfall suggests conditions are not ideal for *Sphagnum* growth and peat formation. If Whim is on the dry side this could exacerbate the effects of N deposition while simultaneously biasing the results leading to an underestimation of the effects, as the control plots against which the N treatments are compared could be under-performing due to water stress. Under-performing, based on the results of the studies presented here, is interpreted as above average respiratory C losses. Thus in order to extrapolate from these results to other Scottish sites, where *S. capillifolium* forms a key component of the bog vegetation, additional gas exchange and capitulum N concentration measurements, which have been shown to be reasonably well correlated with N deposition (Lamers *et al.* 2000), at a range of sites would be recommended for comparative purposes.

Recognising that the Whim N manipulation is a standalone study in Scotland, although it has much in common with the Mer Bleue N manipulation experiment in Canada (similar treatment doses though different timings, different temperature scenarios and some shared vegetation), and that the site is on the dry side for the bogs in Scotland what can be learned from Whim? The CO₂ flux measurements have provided new information that helps explain some of the cover changes, suggesting that hummock forming *Sphagnum* are N sensitive. Importantly the study also suggests there is a memory effect associated with N, so that over time even more modest N loads reduce productivity. The combined N loading comprising the low N dose at Whim was $\sim 16 \text{ kg N ha}^{-1} \text{ y}^{-1}$, which did cause negative effects which over decades may consolidate and significantly affect the cover and performance of *Sphagnum*. However, given that the N loading to most Scottish bogs has and continues to be modelled at $< 10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ with no domination by reduced N (Fig.

8.1., Bealey B., pers. comm.) and the bogs receive high rainfall, the results from this study suggest that provided the critical load for bogs is not exceeded, current levels of N deposition are not a significant threat to the survival of *Sphagnum*. What happens to the C balance over time as result of elevated N, is a question beyond the scope of this single site study.

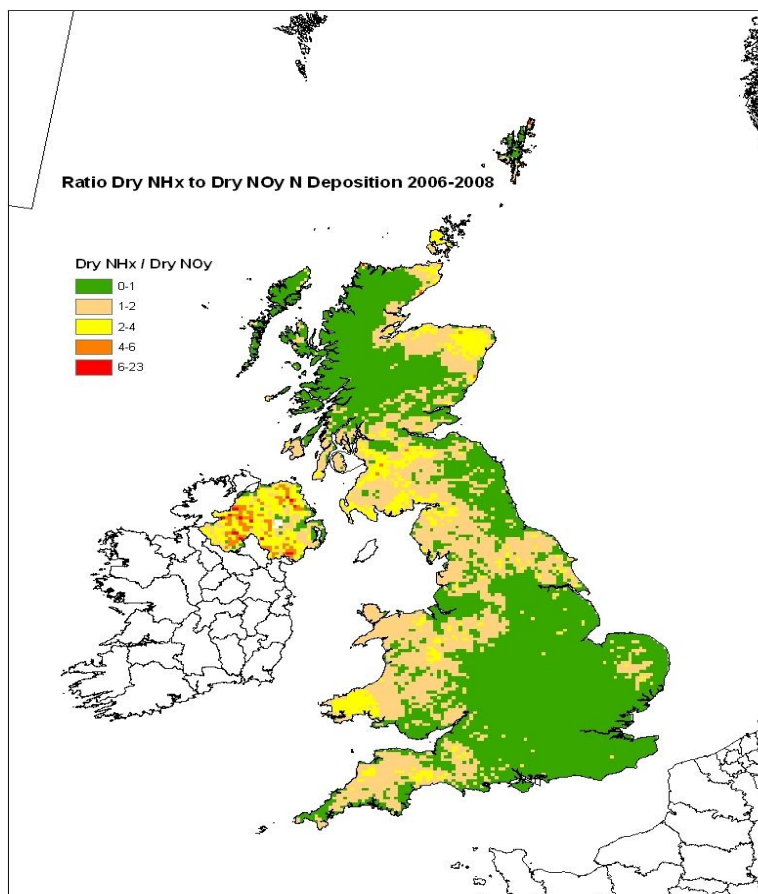


Figure 8.1. N deposition as oxidized and reduced N in the UK ($\text{kg ha}^{-1} \text{y}^{-1}$).

8.6. Possible tools for measuring N impact on *Sphagnum*

There is a need for a simple tool for assessing the N damage in Scottish bogs because there is a legal need for all Scottish bogs to be assessed regarding their state and C accumulation. To be used widely this kind of assessment tool should be very easy to learn and cheap. Based on the results of this study visual assessment could be used for assessment because it correlated very well with the other, measured changes in *Sphagnum* shoot extension and productivity. It would be very easy to learn and

something similar to the Figure 4.1 in Paper I with small written instructions could be used as an aid for the surveyors. Problems with this method could be the subjectivity because it is not a clear technical measurement but a subjective evaluation.

Another, slightly more complex indicator could be *Sphagnum* density. A relationship between %N and *Sphagnum* density was found between the sites and at Whim. This method is more time consuming but more objective. Possible problems with this method could be the difficulty of actually counting the capitula because they are very small and very often under other plants. Although this seems a promising method for assessment it still needs further investigation to make sure that the lower density is the cause of N deposition and high %N instead of being the reason for high %N.

Measurements done at Whim bog showed a linear increase in %N with increasing N deposition (Paper I) which again correlated well with other measurements indicating N damage on *Sphagnum*. Measuring %N could therefore provide a possible tool for measuring N damage. However, measuring %N is very time consuming and raises many questions for the protocol. First problem arises when collecting the samples. In a bog with clear N damage should one sample the healthy looking *Sphagnum* or the ones that look to have visual damage. In sampling one also has to decide which part to sample, the capitulum or the stem part. Also when cleaning the mosses for analysis there are two options: to wash the mosses or not. Washing might remove some of the nutrients that are outside *Sphagnum* but readily available. However, if they are not washed they need to be cleaned with some other method, for example tweezers. This again is very time consuming and it is very difficult to remove all other litter, for example small pieces of other plants. If this method is to be used as a tool for estimating N damage for *Sphagnum* more research needs to be done with the issue because the old estimates might be too high, at least for Scotland.

8.7. Possible management suggestions

If N is an important factor causing peatland degradation, it has to be managed somehow. In areas with high N deposition, adding P and K on a small scale, e.g. to protect endangered species, might be an answer and could be used as a management tool because of the alleviating affect that they have on the effects of increased N (the present study; Limpens *et al.* 2004; Carfrae *et al.* 2007). In addition to conserving endangered species, adding P and K could help keep the productivity of *Sphagnum* high and maintain the C sink function of peatlands (Limpens *et al.* 2004; Williams & Silcock 1997). However, applying P and K can not be used on a large scale because of other problems that might follow from large additions of P and K, for example eutrophication. According to the results from this study, it can also alter the species composition and favour other species over *Sphagnum*.

Although N has been found to cause peatland degradation, it has been also found that they can recover when the N source is removed. Only very little research has been done on the subject but Limpens *et al.* (2003a) found out that the vegetation at least in raised bog seems to be able to recover its nutrient poor state after the source of enrichment is taken away. However, this presumes that the damage caused by the increased N has not been too severe, i.e. that the plants are still alive when the N deposition decreases. Further research is needed on the subject to see if restoring peatlands after damage caused by N deposition is possible. For restoration to have any possibility of succeeding the N source also has to be removed. Because the sources of nitrate and ammonium are different the measures for reducing the deposition are also different depending on which form is being targeted. Ammonium mainly comes from point sources, e.g. farms, whereas nitrate is mainly produced in industry and vehicles. Because the differences between the forms were fairly small both forms should be targeted equally to prevent increased harm for bogs.

8.8. Conclusions

- Both study years were very wet making the conditions ideal for *Sphagnum* and therefore the negative effects found in this study will be probably exacerbated on a dry year.
- The study species, *Sphagnum capillifolium*, has been found in other studies to be less sensitive to N deposition than other hummock species; therefore N thresholds based on this study provide an upper estimate for tolerance which would probably need to be lowered for other hummock species.
- According to the results the threshold for changing the effects from positive to negative for *Sphagnum* productivity, if a threshold exists, is $<16 \text{ kg ha}^{-1} \text{ y}^{-1}$; therefore the results from this study support the current Critical Load estimated for ombrotrophic bogs ($5\text{-}10 \text{ kg ha}^{-1} \text{ y}^{-1}$).
- Long-term N additions decreased the shoot extension and productivity already at relatively small doses of $<16 \text{ kg ha}^{-1} \text{ y}^{-1}$ but no form effect was found; adding PK simultaneously with N alleviated the detrimental effects of N on shoot extension.
- Results from visual assessment correlated well with the decline in shoot extension and productivity and also showed the improvement with PK.
- Increased N deposition led to a decrease in decomposition; N form or dose had no effect and neither did adding PK with N.
- N additions increased N in pore water but no difference was found in DOC between the treatments.
- N additions increased CO_2 flux; unexpectedly nitrate had worse effect than ammonium, this was similar whether PK was added with N or not
- CO_2 formed by far the largest part of the C budget of the *Sphagnum* patches; accordingly, the plots as a whole were sources of C to the atmosphere.
- Possible tools for measuring damage to *Sphagnum* mosses caused by increased N deposition include evaluating visual damage, measuring plant tissue %N or counting the density of the mosses (capitula per m^{-2}) but more research is needed to develop the tools further.

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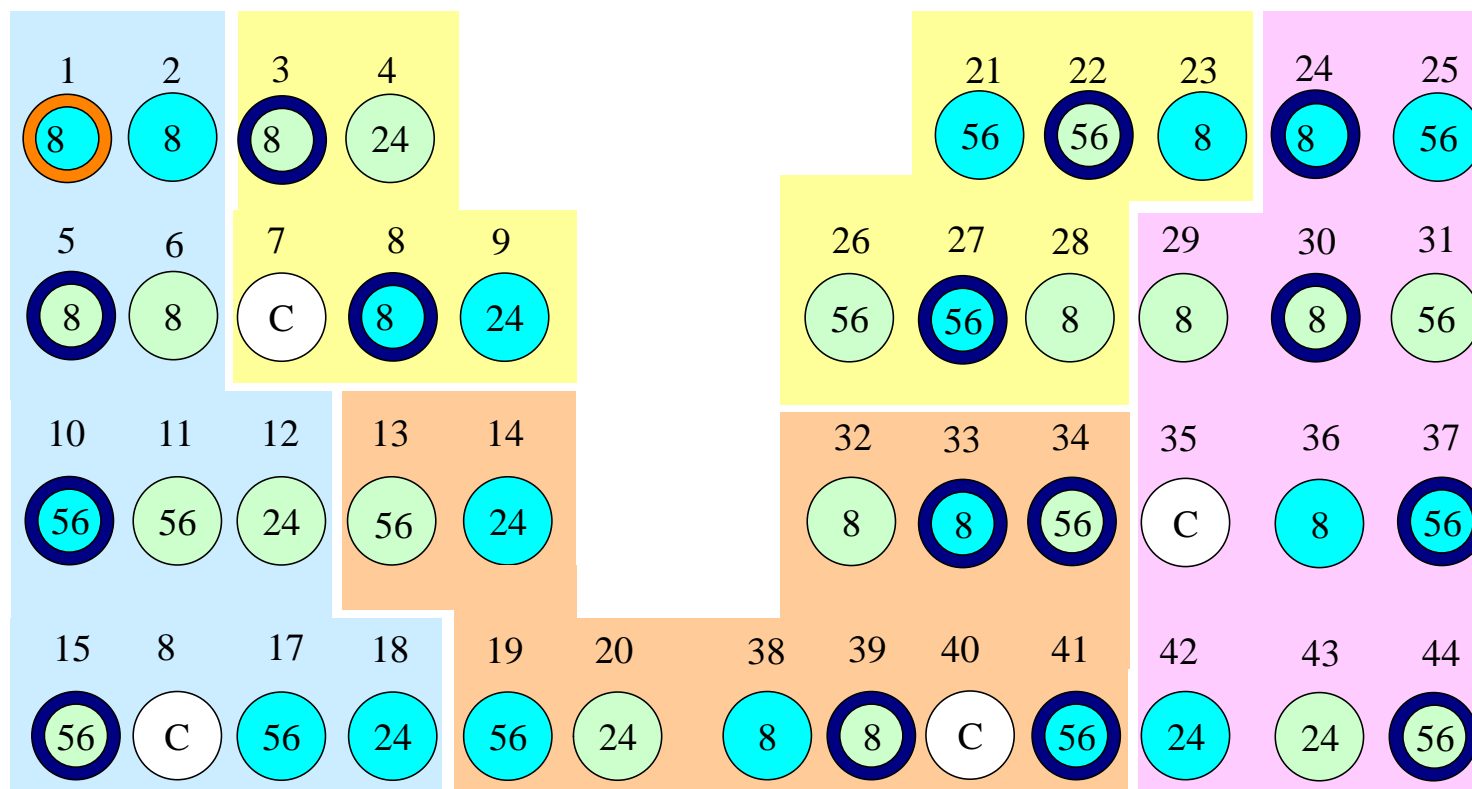
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Appendix I. Treatment plan at the Whim study site.

Whim Bog Treatments



Plot number 1 - 44

Block 1 2 3 4

56 NH₄Cl
56 kg N ha⁻¹ y⁻¹ + PK

24 NaNO₃
24 kg N ha⁻¹ y⁻¹

C Control
8 kg N ha⁻¹ y⁻¹

Appendix II. Summary of previous experiments using the cranked wire method compared to the experimental design in the current study.

Author(s)	Plot size (m ²)	No. of wires	The method of calculating <i>Sphagnum</i> productivity	Brush used to hold the wire on place	Result corrected for change in the capitulum weight if needed
Aldous 2002a	0.25	4	Length*bulk density	No	No
Chapin <i>et al.</i> 2004	3	6	Growth*cover	Yes	No
Gunnarson <i>et al.</i> 2004	0.0225	3	Length*bulk density of the stem fraction	Yes	Yes
Gunnarson & Rydin 2000	0.4	3	Length*bulk density of the stem fraction	Yes	Yes
Limpens <i>et al.</i> 2003b	1	4	n.a.	Yes	n.a.
Aerts <i>et al.</i> 1992	0.4	4	Dry mass increase per individual * plant density	No	Yes
The present study	12.5	15	Stem length growth (cm y ⁻¹)*plant density (individuals per m ²)*dry mass increase (g cm ⁻¹ y ⁻¹)	Corkscrew	Yes

Appendix III. Summary of previous experiments on *Sphagnum* moss decomposition by litter bags compared to the experimental design in the present study.

Reference	Amount of sample per litter bag (g)	Bag placed below the surface (cm)	Stem fraction of <i>Sphagnum</i> used (cm below the capitulum)	Mesh size (µm)	Bag size (cm)	Bag material
Limpens & Berendse 2003	3	-10-15	1-3, 5-7	74	3*7	Polypropylene
Li & Vitt 1997	0.5	-15	below 1 cm	0.8	4*6	Nylon
Rocheftort <i>et al.</i> 1990	0.3-0.8	-10	All	80	2.5*2.5	Nylon mesh
Bragazza <i>et al.</i> 2007	0.5	Just below the surface	2-4	50	-	Polyethylene
The present study	0.2-0.9	-10 – 15	3-8	71	7*7	Polypropylene

Appendix IV. The amount of other plants in the microplots used for measurements in the present study. One + sign means few individuals, two + signs means many individuals either on or around (hanging above) the microplot. ERIVAG = *Eriophorum vaginatum*, CALVUL = *Calluna vulgaris*, ERITET = *Erica tetralix*, HYP = *Hypnum sp.*, PLE = *Pleurozium sp.*, POL = *Polytrichum sp.*, EMPNIG = *Empetrum nigrum*, CLA = *Cladonia sp.*, VACMYR = *Vaccinium myrtillus*, SPAPAP = *Sphagnum papillosum*, VACOXY = *Vaccinium oxycoccos*, ERIANG = *Eriophorum angustifolium*.

Plot	Treatment	ERIVAG	CALVUL	ERITET	HYP SP.	PLE SP.	POL SP.	EMPNIG	CLA SP.	VACMYR	SPAPAP	VACOXY	ERIANG
16.1	C		++										
16.2			+										
16.3		++	++										
16.4			+										
16.5			++										
7.1	C	++	++										
7.2		++	++					+					
7.3													
7.4		++	++										
7.5			+										
40.1	C		++			+							
40.2			++		++			++					++
35.1	C		++										
35.2			++										
35.3							++						
35.4			++		++								
35.5			++										
6.1	NO16	++	++										
6.2		++	++										
6.3		++	++										
6.4		++											
6.5		++	++										
28.1	NO16	++											
28.2		++	+	++									
28.3		+	++			+							
28.4		+	+										
28.5		++	++										
32.1	NO16	+	++										
32.2			+										
32.3			+										
32.4		++	++			+							
32.5		+	++	++									
29.1	NO16	+	++			+							
29.2		++	++										
29.3		+	+										
29.4		++	++										
29.5			++										
12.1	NO32		+										
12.2		+	++			++							
12.3		+	++										
12.4													
12.5			++							+			
4.1	NO32		++										
4.2			++										
4.3		++	++										
4.4		++	++										
4.5		++	++										
20.1	NO32		+										
20.2			+										
20.3		+		+									
20.4			++										
20.5			++										

Plot	Treatment	ERIVAG	CALVUL	ERITET	HYP SP.	PLE SP.	POL SP.	EMPNG	CLA SP.	VACMYR	SPAPAP	VACOXY	ERiang
43.1	NO32		++	+		+							
43.2		+	++	+									
43.3			++		+			+					
43.4		++	+	+									
43.5		+	++	++		+							
11.1	NO64	++	++										
11.2		+	+										
11.3		+	++										
11.4			++										
11.5		+	++										
26.1	NO64	++	++										
26.2			++										
26.3			++										
26.4			++			+							
26.5		++	++	++									
13.1	NO64	++	++										
13.2		+	++										
13.3		++	++										
13.4			++										
13.5			++										
31.1	NO64	++		++									
31.2													
31.3		++											
2.1	NH16					+							
2.2			++										
2.3		++	++										
2.4			++			+							
2.5			++										
23.1	NH16	++											
23.2		++	++			+							
23.3		++	++										
23.4		++	++										
23.5		++	+										
38.1	NH16		++	++									
38.2			++										
38.3				++									
38.4			+										
36.1	NH16	++	++	++									
36.2		++											
36.3			++			++							
36.4			++			++							
36.5		++	++										
18.1	NH32	++	++	+									
18.2		++	++										
18.3			+										
18.4			++										
18.5		+	++										
9.1	NH32	+	+										
9.2		++	++										
9.3			++						+				
9.4			++										
9.5			++										
14.1	NH32	++	++										
14.2		++	++										
14.3		++	++						++				
14.4			++										
14.5		++	++										
42.1	NH32	++	++										
42.2		++	++										
42.3		++	++										
42.4		++	++			++						+	
42.5		++	++										

Plot	Treatment	ERIVAG	CALVUL	ERITET	HYP SP.	PLE SP.	POL SP.	EMPNG	CLA SP.	VACMYR	SPAPAP	VACOXY	ERiang
17.1	NH64		++										
17.2			++										
17.3			+										
17.4		++	++										
17.5			++										
21.1	NH64		++										
21.2			++										
21.3		++	++										
21.4		++	++										
21.5		++	++										
19.1	NH64	+	++										
19.2		+	++										
19.3		++											
19.4		++	++										
19.5			++										
25.1	NH64	++	+			+							
25.2		++	++	++									
5.1	NO16PK												
5.2		+	++										
5.3		++	++										
5.4		++	++										
5.5		+	++			++							
3.1	NO16PK	+	++			+							
3.2			+										
3.3		++	++										
3.4			++			+							
3.5		++											
39.1	NO16PK		++										
39.2			+										
39.3			++			++							
39.4			++			+							
39.5			++										
30.1	NO16PK	+										+	
30.2		+											
30.3			++										
30.4			+										
30.5		++	++	++									
15.1	NO64PK		++										
15.2			++										
15.3			+										
22.1	NO64PK	++											
22.2		+	+										
22.3		++	++										
34.1	NO64PK	++	++										
34.2		++	++										
34.3			++										
44.1	NO64PK	++	++										
44.2			++	++	+								
1.1	NH16PK												
1.2			++										
1.3			++										
1.4		++	++										
1.5			++			+							
8.1	NH16PK		++										
8.2		+	++										
8.3		++	++										
8.4			++										
8.5		++	++										

Plot	Treatment	ERIVAG	CALVUL	ERITET	HYP SP.	PLE SP.	POL SP.	EMPNG	CLA SP.	VACMYR	SPAPAP	VACOXY	ERiang
33.1	NH16PK		++										
33.2		++	++										
33.3		++	++										
33.4		++	++										
33.5		+	++										
24.1	NH16PK	++				+							
24.2		++	++										
24.3			++			++							
24.4		++	+			+							
24.5		++				+							
10.1	NH64PK												
10.2		+	+										
10.3													
10.4													
10.5			+										
27.1	NH64PK	+	+										
27.2		+	++										
27.3			++										
27.4		+	++										
27.5		+	++										
41.1	NH64PK		++										
41.2		++	++										
41.3			+										
41.4			++										
41.5			++										
37.1	NH64PK	++	++				++						
37.2			++										
37.3			++	++									
37.4			++										
37.5													

Appendix V. A paper published in Farrell, C. & Feehan, J. (eds.) *Proceedings of the 13th international peat conference. After wise use – the future of peatlands*, 724 p.

The form of reactive nitrogen deposition is important for the provision of ecosystem services

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Abstract

- Reports effects of manipulating the form of N deposition to an ombrotrophic bog, Whim bog, since 2002. Ammonia was provided by free air release in response to wind direction and windspeed and wet deposited nitrate and ammonium were supplied as and when there was sufficient rainfall, increasing the background deposition of $8 \text{ kg N ha}^{-1} \text{ y}^{-1}$ by 2, 4 and 8 times.
- Ammonia significantly reduced the cover of *Calluna*, moss and lichen leading to an increase in *Eriophorum vaginatum*.
- Wet deposition generally increased *Calluna* cover and reduced the cover of *E. vaginatum*, though not significantly overall and, with the exception of *Hypnum jutlandicum*, the mosses declined with increasing N dose.
- The ammonia driven loss of higher and lower plant cover reduced the sink for N leading to an increase in pore water nitrate, which led to a significant loss of N as the greenhouse gas N_2O .
- The loss of key peatland species leads to the system becoming increasingly N leaky.
- The ammonia driven destruction of the main vegetation cover, leaving only *E. vaginatum*, has reduced productivity and potentially, carbon sequestration.
- The equivalent nitrogen deposition in precipitation has caused some damage to the moss layer, but so far the higher plant cover has not visibly changed even after 7 years. This is consistent with the proportionally tiny increases in pore water nitrate and nitrous oxide flux, by comparison with those mediated by equivalent N doses as ammonia.

- Deposited N retention by *Sphagnum* and other mosses declines between 8 – 16 kg N ha⁻¹ y⁻¹.
- Nitrogen deposition, especially as ammonia-N, can reduce the value of vegetation diversity and thus the conservation value of peatlands. The sustainability of peatlands will be compromised through decline in the keystone species, *Sphagnum*.

Introduction

Peatlands sustain themselves and grow through carbon sequestration (Charman 2002), which in today's world, means they provide a vital ecosystem service. If decomposition / mineralization rates start to exceed productivity, gradually their functionality and land cover will decline. Recently, shrinkage and reductions in peat depth have been occurring in response to anthropogenic activities. In the UK, peat accumulation was estimated to be 0.7 Mt C y⁻¹, with losses due to drainage and peat extraction of ~ 0.5 up to 1.0 Mt C y⁻¹ (Cannell et al 1999). The biogeochemistry of peatlands is highly regulated within a relatively 'self contained cycle' (van Breeman 1995). Pristine bogs are N limited (Aerts et al 1992). Nitrogen is a key plant and microbial growth nutrient, with the potential to both increase carbon assimilation (Marschner 1995) but also change decomposition rates (Charman 2002), although in which direction remains uncertain. However, the responses of peatlands to specific anthropogenic drivers such as enhanced N deposition, especially with respect to the form of N, and climate change, are poorly understood. Given the consequences of increasing N availability are tightly coupled to the biogeochemical cycling and sequestration of carbon via growth, which also drives changes in species composition and function, it is important to improve our understanding of N deposition effects on peatlands.

Peatlands are widespread in northern regions of Europe, areas with low levels of N deposition, often well below 5 up to 10 kg N ha⁻¹y⁻¹, the maximum N Critical Load for bogs, so should we be concerned about enhanced nitrogen deposition? Evidence suggests that sensitivity to N is strongly determined by historical and current N deposition (Emmett 2007), in tandem with the environmental factors that constrain

carbon assimilation, growth rates and species composition. Peatlands generally occur in environments that restrict growth, are cold, wet and acidic with low levels of available nutrients. The vegetation has evolved to tolerate low nutrient availability, developing the ability to economise on nutrient use rather than being able to up regulate C assimilation (Aerts 1999). Thus peatland vegetation is poorly equipped to respond to elevated nitrogen deposition: their biodiversity and function and the services they provide may well be threatened by even very modest increases in their nitrogen deposition. In the context of peatland ecosystems that have evolved over millennia, the increases in reactive nitrogen deposition observed over recent decades (Reay et al 2008) represent a huge stepwise change, even if the N dose appears very small. Because the carbon balance at these sites is particularly important in buffering rising CO₂ levels and because of their potential nitrogen sensitivity, investigating nitrogen effects on carbon processing in peatlands is timely.

This paper reports some key responses, caused by manipulating the form of nitrogen deposition to a peatland ecosystem, Whim bog in the Scottish Borders, which influence the delivery of ecosystem services. Nitrogen deposition in the dry reduced form (ammonia) and wet deposition as either oxidised or reduced N (NaNO₃ and NH₄Cl respectively) has been enhanced over the last 7 years. Using realistic deposition scenarios, where wet deposition is coupled to precipitation patterns, and dry deposition to wind direction, N deposition has been increased by 2, 4 and 8 times the background N deposition of 8 kg N ha⁻¹y⁻¹.

Methods and Site Details

Whim bog, 17 km south of CEH Edinburgh, represents a transition between a lowland raised bog and a blanket bog, 280 m a.s.l., receiving a mean annual rainfall of >900 mm and a mean annual temperature of 8.2 °C. The experimental site at Whim has low background N and S inputs, < 8 kg N (split evenly between wet and dry, reduced and oxidised N), and 4 kg S ha⁻¹ y⁻¹. The vegetation is unmanaged, NVC M19 *Calluna-Eriophorum* – (Rodwell 1991). The surface undulates by up to 0.5 m over 3 to 6 m of acid peat, pH 3.8 (H₂O), base saturation =10%, bulk density between 0.06 and 0.12 g cc⁻¹ and a CN ratio of 33.

The automated free air ammonia release is controlled by a Campbell 23X data logger which records timing, duration and environmental conditions prevailing during NH₃ release (Leith et al 2004). The system is activated when windspeed exceeds 2.5 m s⁻¹ and direction (sonic anemometer) points down the transect. Gaseous NH₃, from a pressurised anhydrous NH₃ cylinder, flows via a mass flow controller through a stainless steel pipe at 3.3 g min⁻¹ into a fan unit where it is mixed with air. The diluted NH₃ is released from a 10 m perforated pipe, one metre from the ground. Monthly mean, but not peak, NH₃ concentrations are quantified along the free air release transect using ALPHA (Adapted Low-cost Passive High Absorption) samplers, 0.1 m above the vegetation, exchanged monthly for regenerated badges and the NH₃ concentration measured (Tang et al 2001). Ammonia concentrations are converted to N dose taking into consideration plant community type, windspeed, surface wetness and using a concentration dependent deposition velocity (Cape et al 2008). The wet deposition is provided from a spinning disc, mounted at ~ 20 cm above canopy height, in the centre of each plot (Sheppard et al 2004a). Treatments are supplied to 4 replicated 12.8 m² plots, at a maximum of 4 mM concentration, as and when sufficient rainfall is collected. Thus, the timing, frequency and N concentrations of the wet and dry treatments differ from each other, as they do in the 'real' world.

Vegetation cover was recorded by species in these large plots, after treatments had been running for > 7 years. Along the ammonia transect the cover was assessed in two 2*1 m² quadrats running parallel to the ammonia source. Nutrient N concentrations were measured (CN analyser) in the 2008 annual increment of the commonest mosses, *Sphagnum capillifolium*, *Hypnum jutlandicum* and *Pleurozium schreberi*. The annual mass increment was also measured using a modification of the cranked wire method, where moss mass that grew above (*Sphagnum*), through (*Hypnum* and *Pleurozium*) a reference level, was harvested after one year. Nitrous oxide (N₂O) emissions were measured using static chambers, (40 cm diameter), based on 4 air samples removed over a 30 minute period in to Teflon bags. The N₂O

concentration was measured with a gas chromatograph (ECD, electron capture detector at 350 °C). The values presented represent mean values (n=4) from October 2008 to October 2009. Soil water (NH_4^+) and NO_3^- concentrations were measured in water sampled from the 0-10 cm layer of peat using suction samplers (mini rhizons 20 ml syringe capacity, filter size 0.45 μm , length 10 cm, inserted at 45°). Values represent the mean of 4 replicates from May 2006 to May 2008.

Results and Discussion

The significance of N form with respect to the effects of enhanced nitrogen on this ombrotrophic bog ecosystem was highly visible. Equivalent N doses of dry ammonia gas, when compared with the same nitrogen doses applied in rain water, caused damage within 1 year, on a scale that has still not been replicated, 7 years later in the wet plots, even with 56 kg N ha⁻¹y⁻¹. The common matt forming lichen *Cladonia portentosa* was killed at NH_3 -N deposition equivalent to the 56 kg N ha⁻¹y⁻¹ in < one year (Sheppard et al 2004b). Similar levels of death were also seen amongst *Calluna* (Sheppard et al 2008) and *S. capillifolium* (Sheppard et al 2009). By comparison *Eriophorum vaginatum* (cotton grass) has increased its cover along the ammonia transect, compared to the wet treatment plots where there appears to be no obvious change, though a trend toward decreasing cover.

In the presence of high ammonia - N doses, > 4 times the ambient N deposition to the site, the cover of all the *S. capillifolium* and pleurocarpous mosses, *H. jutlandicum* and *P. schreberi* has fallen to <12 %, < half the pre-treatment cover levels. Both *S. capillifolium* and *P. schreberi* have also responded negatively, *i.e.* lower cover values, to the N dose in wet deposition, but the loss of cover was independent of N form. Loss of cover was proportionally much smaller than found with ammonia for *S. capillifolium*. For *P. schreberi* cover is negatively related to N dose being most pronounced in the high reduced N treatment. Growth measurements confirm the potency of N for *P. schreberi* except at the lowest input. Interestingly the other pleurocarpous moss *H. jutlandicum* appears to be tolerant of wet oxidised N and even stimulated by the high dose of nitrate.

Calluna appears to be indifferent to both N dose and N form in wet deposition but $\text{NH}_3\text{-N}$ deposition of $> 16 \text{ kg N ha}^{-1} \text{ y}^{-1}$ has dramatically reduced *Calluna* cover along the ammonia transect. By contrast *E. vaginatum*, likewise indifferent to N form and dose in wet deposition responded positively to high ammonia and the consequent reduction in *Calluna* cover, which accounted for 71 % of the variation in cover of *E. vaginatum* growing along the ammonia transect. *Calluna* appears to be controlling the cover of *E. vaginatum*, which can tolerate both high ammonia concentrations N dose and the increase in soil pH.

Peatland ecosystems such as Whim bog evolve under low nutrient availability, thus enhancing reactive N deposition, the second most important plant growth nutrient behind carbon, will inevitably change this ecosystem. The issues to address here are associated with establishing whether all forms of N cause similar effects, whether these effects are detrimental, how the effects impact on ecosystem services and which parameter to adopt as metrics of effect, change?

We have shown that the relationship between component species has changed in response to increasing the supply of mineral N and that N form as dry deposition drives responses, per unit N deposited, much faster than wet N deposition. The change in dominance from *Calluna* to *E. vaginatum* in response to ammonia so far shows no sign of being replicated under the wet treatments. *Calluna* cover only accounts for 34% of the variation in *E. vaginatum* cover in the wet plots and while *Calluna* cover has increased over time, not significantly in response to N treatment per se, *E. vaginatum* cover has declined, though again not significantly. Sheppard et al (2008) suggested the ammonia driven death of *Calluna* was mediated via interaction between ammonia effects and a 'normal' stressing agent for *Calluna* e.g. winter desiccation: ammonia appeared to impede stomatal closure, causing stomata to close at a much lower water content in plants subject to high ammonia concentrations. Although wet N deposition does increase winter desiccation the effects generally don't lead to the loss of this species as they have with ammonia, since a significant proportion of the canopy recovers (Carroll et al 2000). The irreversible ammonia damage to *Calluna* was associated with a significant, $> 30\%$

increase in foliar N, increases in foliar N with wet deposition are still modest by comparison (Sheppard et al 2008). These results suggest that where *Calluna* remains dominant *E. vaginatum* cover is naturally suppressed and that until wet deposited N starts to restrict the growth of *Calluna* this balance is sustainable. Cotton grass provides a conduit for methane, restricting the likelihood of methane oxidation and increasing the potential for methane emission (Greenup et al 2000). Increases in its cover will have implications for C loss, and greenhouse gas emissions.

Loss of moss cover, most devastating with ammonia, but also significant with wet N $> + 8 \text{ kg N ha}^{-1} \text{ y}^{-1}$ can have consequences for N sequestration. Mosses are accomplished at sequestering most of the N in wet deposition when it is supplied in small doses (Lamers et al 2000) however, this ability declines sharply with increasing N deposition and species effectiveness varies. In the control plots *Sphagnum* continues to absorb all the ambient deposition, with *P. schreberi* sequestering $> 90\%$ and *H. jutlandicum* least, at $\sim 40\%$. Above the annual deposition the 'natural filter' begins to fail (Nordbakken et al 2003). *H. jutlandicum*, which is the most N tolerant species, sequesters least N, but sequesters proportionally more than the other mosses at high N inputs. These results support the many ^{15}N labelled studies that show the significance of mosses for N sequestration, providing the N input remains below 5 to 10 $\text{kg N ha}^{-1} \text{ y}^{-1}$ (Nordbakken et al 2003, Curtis et al 2005).

The repercussions of the reduced 'N sequestration' efficiency of the moss flora has likely contributed to the increases in soil water nitrate and increased risk of N loss by denitrification and leaching, which can be associated with surface water acidification (see Curtis et al 2005). In this study large N losses have, so far, only been recorded in response to ammonia. Only small increases in soil water N concentrations in relation to wet N dose, with the highest concentrations in the reduced N treatments. These differences most likely reflect the increase in the *Calluna* sink for N in these wet N plots. At N doses below $24 \text{ kg N ha}^{-1} \text{ y}^{-1}$, there were no significant differences between the N forms, wet or dry, or reduced versus oxidised, again most likely reflecting in particular the relatively smaller impact of the lower ammonia N dose on the vegetation N sink. In response to wet N deposition N

losses through denitrification were small. The high N treatment as ammonia leaked almost 8 times as much N, compared with the wet N treatments, with ~ 30 % in the form of nitrate, indicating a high level of nitrification in response to ammonia N deposition. There was a significant 0.5 unit increase in soil pH in this treatment (data not shown). These two factors, together with the loss of moss and *Calluna* cover, and the relatively small uptake of mineral N by *E. vaginatum* (data not shown) probably explain the relatively high N₂O flux with dry but not wet N deposition.

These results show that N additions compromise the ecosystem services that ombrotrophic bogs / peatlands perform but also demonstrate that the form in which N deposits and not just the N dose is important. With respect to N dose we have found, that effectiveness of the N sequestration capacity of *S. capillifolium* occurs at similar N doses, irrespective of the form of N in wet deposition, to those suggested by Lamers et al (2002) and Blodau et al (2006) using ¹⁵N, ~ 15 – 20 kg N ha⁻¹y⁻¹. The estimated N content of the *S. capillifolium* exceeded the ambient deposition at the site, suggesting the *S. capillifolium* supports a significant N fixing cyanobacterial community, which can contribute > several kg N ha⁻¹y⁻¹ in *S. capillifolium* (Markham 2009). By contrast dry N deposition has not just compromised the ability of the vegetation to sequester N, it has caused the physical loss of this sequestering media, exposing the bare peat.

The difference between dry and wet N deposition for ecosystem services *i.e.* immobilising N deposition so that it does not leak into water or the atmosphere, was mediated by their respective effects on the amount and quality of the vegetation. Ammonia has killed all the vegetation leaving only *E. vaginatum* a deep rooted *Cyperaceae* and some acrocarpous mosses. *E. vaginatum* has a low reliance on mineral N (Nordbakken et al 2004), although it can be a strong competitor for nitrate, which is a prerequisite for denitrification (Silvan et al 2004). The loss of the vegetation sink on bogs such as this, in response to ammonia leads to a surplus of nitrate and it appears that in the presence of this electron acceptor, other conditions are suitable for supporting significant levels of denitrification and nitrous oxide production. Where the cover is dominated by *Calluna*, which restricts *E. vaginatum*,

and the moss layer is maintained, more of the N is retained and less is available in the pore water for the microbes responsible for denitrification to utilise.

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Appendix VI. Paper published in Farrell, C. & Feehan, J. (eds.) Proceedings of the 13th international peat conference. After wise use – the future of peatlands, 724 p.

CARBON AND NITROGEN CYCLING IN SPHAGNUM CAPILLIFOLIUM IN RESPONSE TO NITROGEN DEPOSITION AND FORM

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Summary: Increased nitrogen deposition can change the rate of production and decomposition of *Sphagnum* mosses in bogs and thus affect their carbon sink function. This study shows results from an N manipulation experiment in southern Scotland, which has been running for six years, and where different N doses and N forms are added to a bog ecosystem. The productivity and decomposition of *Sphagnum capillifolium* are being studied with cranked wires and litter bags. First results from these experiments show a decrease both in productivity and decomposition with increased N deposition. However, adding P and K alleviates these effects thus suggesting that the ecosystem is turning from N-limited into P- or K-limited.

Keywords: Decomposition, Nitrogen (N), Productivity, *Sphagnum capillifolium*, Whim

Introduction

Only 3% of all the peatlands of the world are classified as blanket bogs; The British Isles are an important reserve having 10 to 15 % of them (Tallis *et al.* 1997). In ombrotrophic bogs the morphological and physiological properties of *Sphagnum* mosses create nutrient poor and acidic conditions, and thus perpetuate the already nutrient poor conditions (Van Breemen 1995). *Sphagnum* mosses also take up most of the atmospherically deposited N and thus store large amounts of N in their slowly decomposing tissues making it unavailable for vascular plants (Aerts *et al.* 1992). The increased atmospheric N deposition in recent decades can lead to higher N concentrations in *Sphagnum* tissues and so cause nutrient imbalance or even be toxic (Gunnarsson and Rydin 2000; Hoosbeek *et al.* 2002; Bragazza *et al.* 2005). This makes the *Sphagnum* carpet lose their ability to retain all deposited N which then leaches to the rhizosphere (Aerts *et al.* 1992; Limpens *et al.* 2003; Bragazza *et al.* 2005). It can also affect their productivity and/or decomposition and thus possibly lead to a decrease in the carbon (C) sink function of peatlands.

Increasing reactive N availability can lead to increasing productivity of *Sphagnum* mosses at least in short term and in small doses (Aerts *et al.* 1992; Vitt *et al.* 2003). However, it seems that when the N deposition becomes too much, the productivity does not increase anymore and may start to decrease (Aerts *et al.* 1992; Gunnarsson and Rydin 2000; Aerts *et al.* 2001; Limpens *et al.* 2003; Vitt *et al.* 2003). It is also possible that other nutrients or changes in the nutrient ratios are responsible for changes in the growth of *Sphagnum* rather than N alone, especially in the high N deposition areas where other nutrients may become limiting (Aerts *et al.* 1992; Aerts *et al.* 2001; Hoosbeek *et al.* 2002; Limpens *et al.* 2003). Potential decay rates of *Sphagnum* litter are strongly controlled by N and P availability (Aerts *et al.* 2001). The higher N concentration in the *Sphagnum* tissue compared to C and P produced by increased N deposition can lead to faster decomposition (Limpens and Berendse 2003). However, a study by Hoosbeek *et al.* (2002) showed that increasing N in the litter can lead to shortage of P or K and thus limit the decay.

The aims of this study are to: 1) compare the effects of different N loads and N forms (reduced and oxidised) on *Sphagnum capillifolium* productivity and decomposition and, 2) to examine how P and K additions influence the effects of increased N.

Material and Methods

The experiments are being carried out at Whim bog, which is situated c. 30 km outside Edinburgh, Scotland (3°16'W, 55°46'N). It represents a transition between a lowland raised bog and a blanket bog and is situated 280 meters a.s.l. The annual rainfall in the area is 900 mm and the mean annual temperature 8.8 °C. The background N deposition is relatively small, approximately 8 kg ha⁻¹ y⁻¹, which makes it a good site to study the effects of increased N deposition because the effects on control plots are relatively small.

This project makes use of an N manipulation experiment carried out at Whim bog where the effects of different N loads and forms have been studied since 2002 (Sheppard et al. 2004). There are all together 44 plots, which are 12.5 m² in size and situated 3 m apart to avoid contamination from the next plot. N is added to the plots either as nitrate (NaNO₃) or ammonium (NH₄Cl) with doses of 8, 24 and 56 kg ha⁻¹ y⁻¹ on top of the background deposition with sprinklers year round with rain water during rain events. There are also plots with N load of 8 kg ha⁻¹ y⁻¹ and 56 kg ha⁻¹ y⁻¹ where P and K are added as K₂HPO₄ with N. The treatments add 10% of water to the rainfall volume. There are 4 replicates of every treatment and four control plots, which only receive the additional rain water. For the experiments five microplots of *Sphagnum capillifolium* were chosen from every study plot. (See also Sheppard *et al.* in this issue)

The productivity of *S. capillifolium* is being studied with a modification of the cranked wire method (Clymo 1970) so that the lower part of the wire that was inserted into the *Sphagnum* carpet was twist into a cork screw form to prevent it moving up or down. Three wires were inserted to every microplot in May 2007 and they are being measured every three months. The decomposition is being studied with litter bags. Four bags with 0.3-0.8 g of dry weight moss were inserted into every

study plot in April 2007. The bags are being taken up every six months. In addition, rhizon samplers were inserted into *Sphagnum* to the same layer where the litter bags are incubated and water from these collected every two weeks and analysed for dissolved organic carbon (DOC).

Results and discussion

Growth

Results from the productivity experiment to date show that increased N dose has a detrimental effect on the growth of *S. capillifolium* and that the effect is more pronounced the greater the N dose (Fig 1a). This is in agreement with previous studies (Aerts *et al.* 1992; Gunnarson and Rydin 2000; Aerts *et al.* 2001; Limpens *et al.* 2003; Vitt *et al.* 2003). The effects of reduced N (NH_4^+) are more detrimental, i.e. it decreases growth more than oxidised N (NO_3^-) with the same dose. This suggests that already small doses are affecting the growth in contrast to some studies which have suggested that in small doses the productivity increases (Aerts *et al.* 1992; Vitt *et al.* 2003). However, the studies that found an increase were short-term whereas in this study the N has been added for six years. Adding P and K with N seems to alleviate these effects (Fig. 1a). This suggests that increasing N deposition is changing the *Sphagnum* mosses from N-limited to P- or K-limited as found in other studies (Aerts *et al.* 1992; Aerts *et al.* 2001; Hoosbeek *et al.* 2002; Limpens *et al.* 2003).

Decomposition

The first results from the decomposition study suggest that increased N deposition decreases the rate of decomposition compared to the control plots (Fig. 1b). This is in contrast to the study by Limpens and Berendse (2003) that found an increase in decomposition with increasing N deposition. The decrease in decomposition might be due to change in the limiting nutrient from N to P or K or both, as suggested by Hoosbeek *et al.* (2002) and the growth measurements in this study. This is supported by the slightly increased decomposition in the plots where P and K are added

compared to the plots with only N added, although still lower than in control plots (Fig. 1b).

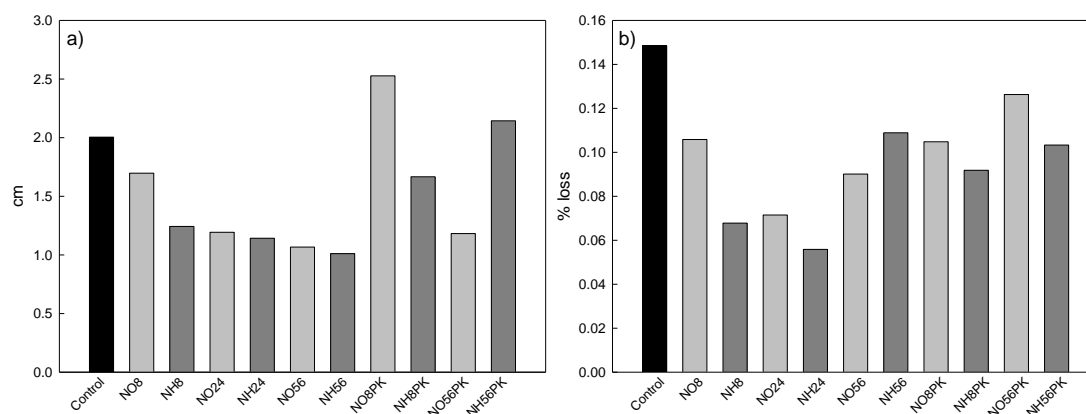


Figure 1. a) The length growth of *Sphagnum capillifolium* in cm between May and November 2007 measured with cranked wires. The bars each represent an average for the 11 different groups. b) The percentage loss of moss material in the litter bags between April 2007 and October 2007. The bars each represent an average for the 11 different groups.

Dissolved organic carbon

There is a general trend in the dissolved organic carbon (DOC) concentrations in the water samples with higher amounts in autumn and the amounts lowering during the winter. In general, the water samples from the NO₃⁻ plots have more DOC in them than water from the NH₄⁺ plots (Fig. 2). This is very pronounced in the plots where P and K are added with N. This suggests a higher level of decomposition in the NO₃⁻ plots which is also evident in the mass loss from the litter bags (Fig. 1b). However, the 8 kg ha⁻¹ y⁻¹ is an exception for this having more DOC in the NH₄⁺ plots.

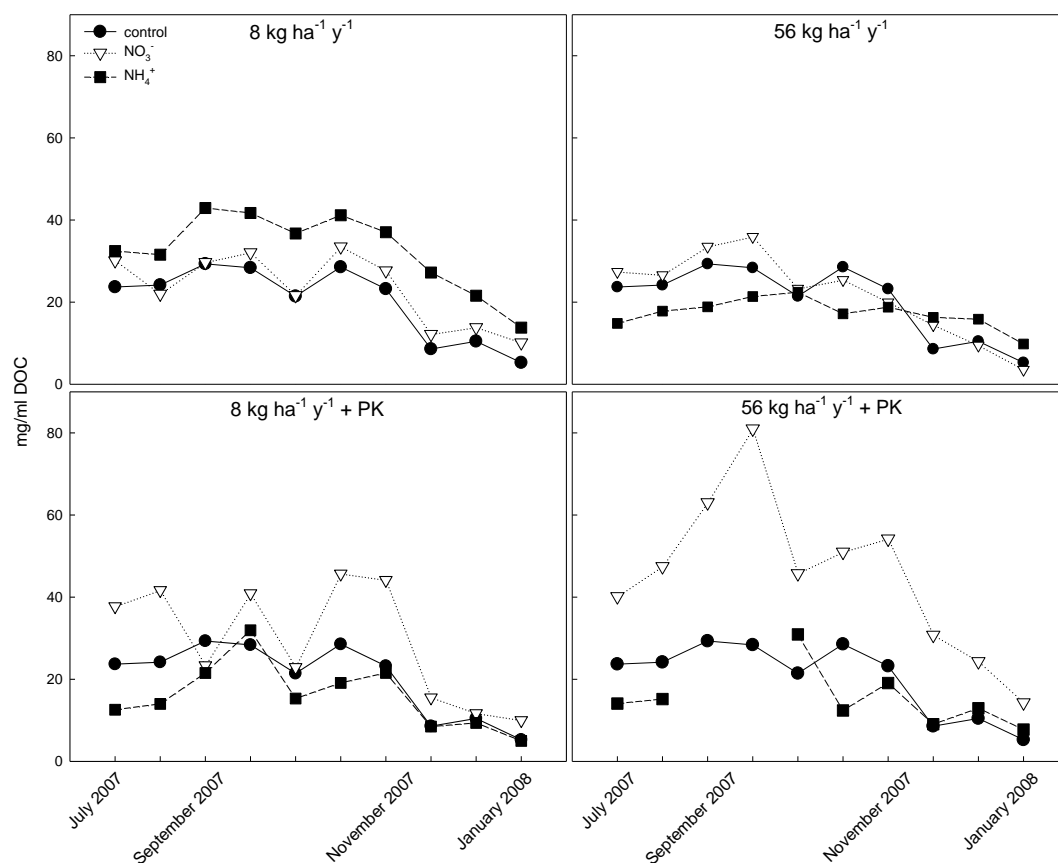


Figure 2. The dissolved organic carbon in mg/ml measured from rhizon samplers placed inside *Sphagnum* in 10-15 cm below the surface. The results are an average from the four replicates for every treatment.

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Appendix VII. A Paper published in *Land Management in a changing environment*, Proceedings of SAC and SEPA biennial conference Edinburgh 26-27 March 2008.

DOES ENHANCED NITROGEN DEPOSITION REPRESENT A THREAT TO SPHAGNUM AND THUS THE SUSTAINABILITY OF SCOTTISH PEATLANDS?

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Summary: Nutrient limited ombrotrophic bogs and peatlands support high conservation valued ecosystems, potentially susceptible to current elevated levels of reactive nitrogen (N) deposition. Here, we present the effects and consequences of different N forms, wet, dry, reduced and oxidised N on the functioning of a bog moss, *Sphagnum capillifolium*. *Sphagnum* mosses maintain the acid, low nutrient conditions, crucial for the sustainability of peat lands, where productivity must exceed decomposition. Dry deposited ammonia substantially elevated shoot N status, which led to tissue breakdown loss of function and death in *S. capillifolium*. Wet deposited nitrate and ammonium also negatively affected *S. capillifolium*, significantly reducing shoot extension and cover and significantly elevating N status. These effects occurred over 5 years and were significant even at the lowest reduced N dose, 8 kg N ha⁻¹ y⁻¹ (background = 8-10 kg N ha⁻¹ y⁻¹), highlighting the threat N poses for the effective functioning of bog ecosystems.

INTRODUCTION

Blanket and raised bogs are peat based ecosystems, with a restricted world distribution, covering around 1.5 million hectares in Great Britain. Peat based bogs

can represent thousands of years of organic matter accumulation and are amongst Britain's most ancient natural/semi-natural ecosystems. Bogs are valued for their specialised plant and bird communities and their ability to act as a sink for carbon. These plant communities are adapted and restricted to nutrient limited conditions sustained by the wet, often anoxic, acidic conditions. These conditions, which restrict decomposition, are generated by the unique properties of the keystone species belonging to *Sphagnum spp.* or 'bog moss' (Clymo and Hayward, 1982). Today, apart from reclamation, drainage for other land uses, one of the major threats to the sustainability of bogs comes from the enhanced deposition of reactive nitrogen. *Sphagnum* mosses are efficient scavengers of mineral nitrogen, this N 'sequestration' while increasing their N sensitivity (Limpens *et al.* 2004), helps to exclude faster growing plant species with higher transpiration rates and the potential to lower the water-table. Without *Sphagnum* many bogs would be transformed into drier grass/tree dominated habitats, at the expense of all the specialized species (Aldous, 2002).

Atmospheric reactive nitrogen comes in the oxidised form from fossil-fuel combustion and in the reduced form from food production, especially via intensive livestock units and fertilizer use. These pollutants can be deposited locally (ammonia) or transported for varying distances in the atmosphere to affect air and rainfall quality across the UK. In Scotland bog and peatland ecosystems are found predominantly in the wetter north and west, and thus receive most of their N load as wet deposition. Bogs can also be affected by nitrogen eutrophication from runoff or local sources of ammonia. In Europe, many bogs occurred in regions of intensive livestock farming, here the detrimental effects of reduced nitrogen, ammonia have transformed such bogs into grassy plains (Heil and Diemont, 1983). The majority of British bogs, with the exception of those in Northern Ireland, by comparison do not occur in areas of high nitrogen deposition, although most sites already receive the Critical Nitrogen Load of 5-10 kg N ha⁻¹ y⁻¹.

Our study compares the *in situ* effects of three different reactive N forms: gaseous ammonia (NH₃), wet ammonium (NH₄⁺) and wet nitrate (NO₃⁻) on an ombrotrophic bog under real world conditions and at realistic concentrations. This paper reports the

effects of these 3 N forms on the growth, cover and N status in a key hummock dwelling *Sphagnum*, *S. capillifolium* and discusses the implications of elevated N deposition for the sustainability of peat land ecosystems.

MATERIALS AND METHODS

In 2002 a unique N manipulation experiment comprising an ammonia fumigation (Leith *et al.* 2004), simulating ~ 20,000 broiler hens (Sheppard *et al.* 2008), and a wet automated spray system (Sheppard *et al.* 2004) was established on Whim bog in the Scottish Borders. The ammonia release was programmed so that gaseous ammonia mixed with air was released over a 100 m long transect, from a 10 m perforated pipe, 1 m above the vegetation, into the prevailing wind, providing a concentration and N deposition gradient. Equivalent N doses to those applied to the wet treatments, (8, 24 and 56 kg N ha⁻¹y⁻¹) were estimated at 8, 16 and 32 m from the NH₃ source (Cape *pers comm.*). Data are reported for these distances. The wet treatments were provided from revolving sprayer heads, in the centre of each plot, as either sodium nitrate or ammonium chloride at 3 N doses: 8, 24 and 56 kg N ha⁻¹y⁻¹, over and above the 8-10 kg N ha⁻¹y⁻¹ background, at a maximum concentration of 4 mM N, to 4 replicate plots per treatment. A water only control was included to assess the effects of the additional 10 % precipitation. Treatments were fully replicated, one 12.5 m² treatment plot per each of 4 blocks and applications were coupled to rainfall, no rain no treatment. Treatments have been applied throughout the year since summer 2002, as and when meteorological conditions permitted.

Species cover was assessed initially in May 2002, in 2004 and in summer 2007 for 3 x 0.25 m² permanent quadrats, subdivided into 16 squares, per plot. The overall percent cover of *Sphagnum* was also estimated by 2 observers independently, for each plot in 2004 and 2007 and reported as % change. *Sphagnum* plot was highly variable and this variability was not replicated in the quadrats, likewise the 4 replicate plots had differing initial amounts of *Sphagnum* and were not replicates in the true sense. The data presented are weighted cover estimates of the degree to which cover in the quadrats has changed over 5 years. Extension growth was assessed between May and November 2007 using 10 modified crank wires (Limpens *et al.* 2004) per plot inserted into clumps of healthy *S. capillifolium*. Plot means (4)

were analysed using GenStat Release 10.1 (ANOVA, General treatment structure, no blocking: model based on N addition, N dose and N form). In 2005 samples of the apical 2 cm of stem were removed, cleaned and frozen, then extracted in ultra-pure water for 4 h, filtered and the soluble NH_4^+ measured using an ammonia flow injection analysis system. Results are presented in $\mu\text{g N g}^{-1}$ dry wt after log transformation.

RESULTS

After 5 years the *S. capillifolium* within 16 m of the NH_3 source had died, and at 32 m only red pigmented shoots survive. No ‘vital’ shoots were available for growth or N status monitoring in the ammonia treatments. In the wet treatment plots, *S. capillifolium* has increased in the control by ~ 20% (Figure 1) but decreased in response to N, particularly reduced N, by ~ -40%, with the greatest effects at 56 kg N $\text{ha}^{-1}\text{y}^{-1}$. Effects of oxidised N were also detrimental, ~ -30%. In the quadrats also, N additions at the highest doses or as reduced N decreased cover, whereas with oxidised N, up to 24 kg N $\text{ha}^{-1}\text{y}^{-1}$ (Figure 2) there was still a small positive effect on cover, albeit substantially less than in the control quadrats.

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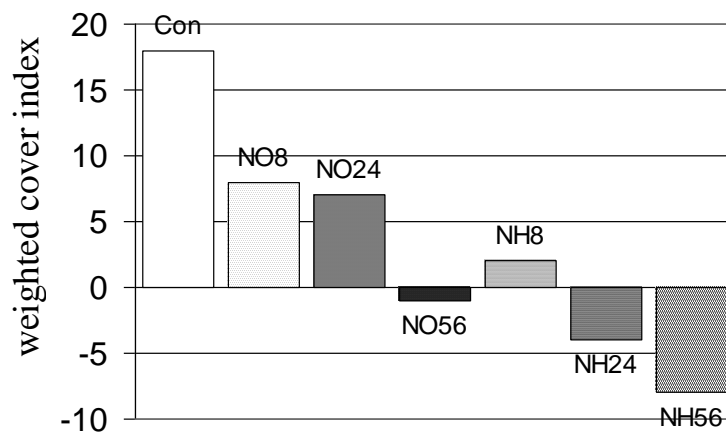


Figure 1. The weighted cover index represents the change in the cover of *Sphagnum capillifolium* between 2002 (pre-treatment) and 2007 in response to wet deposition, applied as a spray, of sodium nitrate (NO) or ammonium chloride (NH) at 8, 24 and 56 kg N ha⁻¹ y⁻¹.

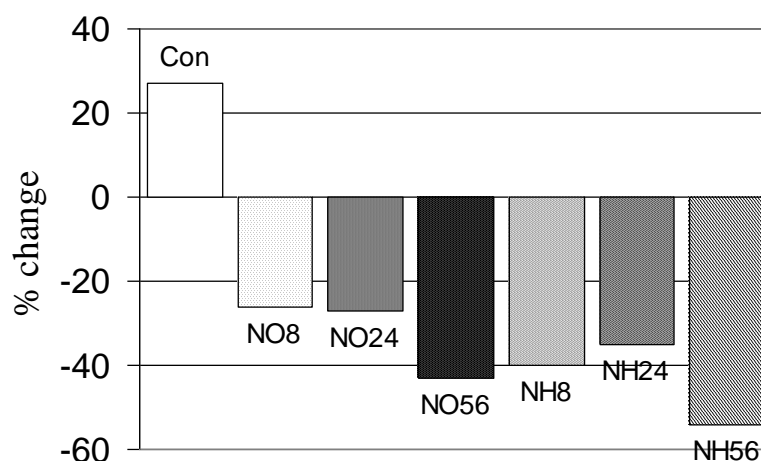


Figure 2. Mean percent change in the cover of *Sphagnum capillifolium* growing in the four 12.5 m² plots, between 2004 and 2007 in response to wet deposition, applied as a spray, of sodium nitrate (NO) or ammonium chloride (NH) at 8, 24 and 56 kg N ha⁻¹ y⁻¹.

When extension growth was measured in the wet plots, after 5 years of continuous treatment, the detrimental effects of N were highly significant ($P=0.003$). Reduced N additions reduced shoot extension, irrespective of dose (Figure 3), even at 8 kg N ha⁻¹ y⁻¹ dose in contrast to oxidised N where the effects were moderated by the dose. The soluble N (NH₄⁺) status of the apical stem section was significantly enhanced in

response to wet deposited N ($P=0.019$) responding to both dose ($P<0.001$) and form ($P=0.099$), with reduced N causing the largest N increase (Figure 4).

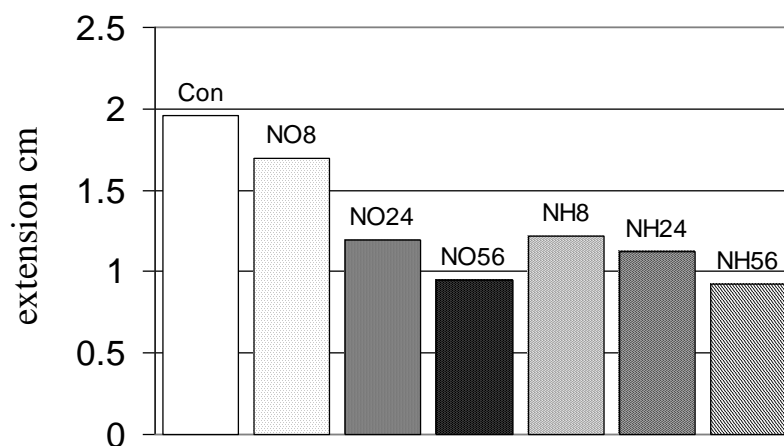


Figure 3. Extension growth in *Sphagnum capillifolium* measured from a fixed position, cranked wire, 10 per plot, between May and November 2007 in response to wet deposition, applied as a spray, of sodium nitrate (NO) or ammonium chloride (NH) at 8, 24 and 56 kg N ha⁻¹ y⁻¹.

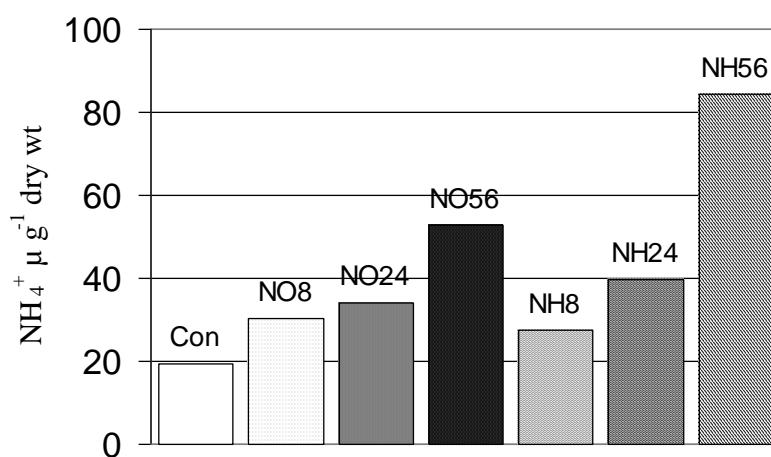


Figure 4. Soluble ammonium in *Sphagnum capillifolium*, apical 2 cm frozen, then extracted in water, in October 2005 in response to wet deposition, applied as a spray, of sodium nitrate (NO) or ammonium chloride (NH) at 8, 24 and 56 kg N ha⁻¹ y⁻¹.

DISCUSSION

This experiment, the first to study the effects of different N forms on an ombrotrophic bog ecosystem has shown conclusively the vulnerability of such ecosystems to elevated N deposition, fully corroborating the low Critical Load adopted for these ecosystems at the UNECE Expert workshop Berne 2002. Critical Loads are set in order to protect ecosystems, providing a temporal protection ~ 20 years to take account of the potential for cumulative effects. The responses reported for Whim bog are important because unlike many previous manipulation studies which were conducted in areas of high background N deposition, and where acclimation to N may have already occurred, the N deposition at Whim is relatively low and more similar to that found where the majority of Scottish bogs occur. At Whim bog where the wet treatment application is coupled to rainfall and N concentrations are relatively low, by experimental standards, although still twenty-fold higher than occult deposition and two orders of magnitude higher than concentrations in rainfall, the ambient deposition is within the Critical Load. In response to ambient N inputs *S. capillifolium* appears quite healthy, maintaining growth rates consistent with other hummock species (Limpens *et al.* 2004) and thus its competitive ability. However, at just double the Critical Load and after only 5 years, deleterious effects of N in precipitation can be identified, especially when the N deposition is dominated by reduced N. The accumulation of potentially toxic NH_4^+ ions may be contributing to the loss of vitality, however, competition from nitrophytes also appears to be important. At Whim *S. capillifolium* is being overgrown and out competed by more N tolerant pleurocarpous mosses such as *Hypnum jutlandicum* and *Pleurozium schreberi*. In addition, the additional N, especially at the lower doses, has increased the growth of ericoid shrubs, which in turn will restrict the light reaching these understorey mosses and potentially increase evapo-transpiration and lower the water-table. The detrimental effects of N on *S. capillifolium* are strongly exacerbated during droughts, even though the absence of precipitation restricts N deposition (Carfrae *et al.* 2007). Likewise, at reduced light levels, the potential to detoxify N via assimilation is reduced.

These results corroborate the accumulating literature regarding N impacts on *Sphagnum*, which also highlight the acute N sensitivity of hummock dwelling *Sphagnum* species (Gerdol *et al.* 2007). *S. capillifolium* is the only constant *Sphagnum* in the M19 (National Vegetation Classification) mire category (Rodwell *et al.* 1991), competing with the hypnaceous mosses for the ground carpet. Given that M19 has already been almost eradicated from south of the Border through a combination of drainage, pollution and erosion, we need to be concerned for this type of mire in Scotland. Comparing the N deposition maps for moorland (closest surrogate for bogs) and the distribution of *S. capillifolium* / M19 (Rodwell *et al.* 1991) reveals that current levels of N deposition are approaching levels, shown here to cause detrimental effects leading to reduced cover, in the south of Scotland, northern England and the Cairngorms. Nitrogen depositing as ammonia gas was the most damaging N form, however, this threat is more restricted to the co- location of bogs and localized NH₃ sources most commonly found in Northern Ireland. This N manipulation study has demonstrated that enhanced nitrogen deposition, at doses in excess of the Critical Load will have a profound negative impact on the hummock forming species *S. capillifolium* the keystone *Sphagnum* moss in *Calluna vulgaris* – *Eriophorum vaginatum* blanket mire. Although, in Scotland the distribution of *S. capillifolium* means that many of its locations receive N deposition at doses below the Critical Load, we also know that changes in the water table level, especially lowering, considerably exacerbate the detrimental effects of elevated N deposition (Carfrae *et al.* 2007). We currently need a better understanding of these interactions, together with the responses of pool inhabiting *Sphagnum* so that we can reduce the uncertainties in predicting the response of *Sphagnum* species and peatlands to the combination of climate change and N deposition.

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